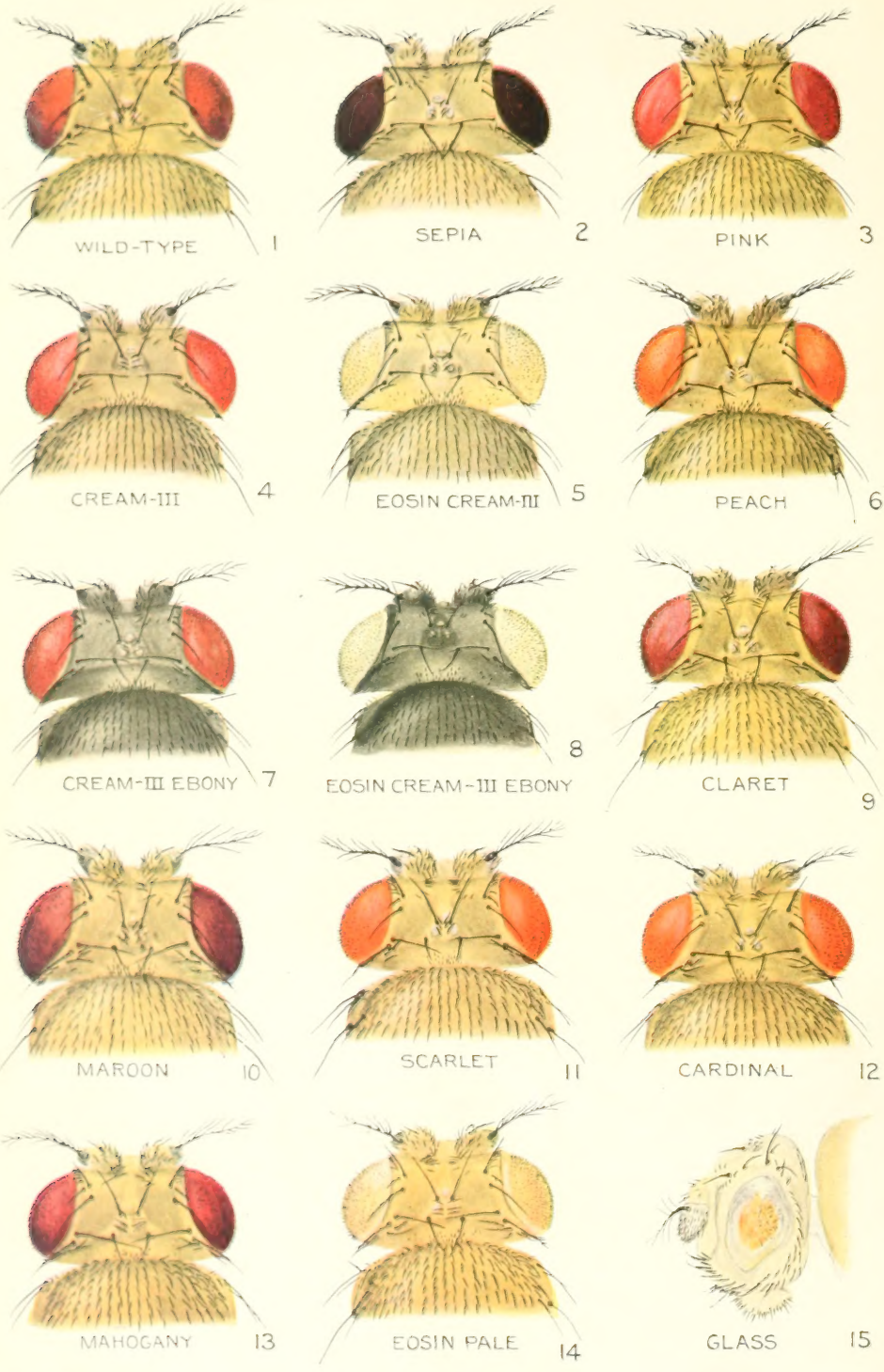
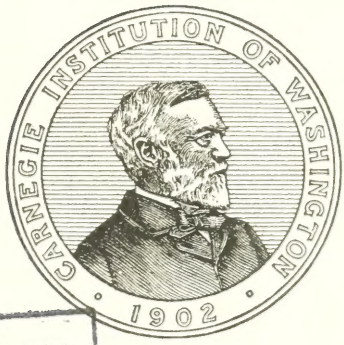


E. M. WALLACE PINX.

THIRD-CHROMOSOME EYE-COLORS OF DROSOPHILA

THE THIRD-CHROMOSOME GROUP OF MUTANT CHARACTERS OF DROSOPHILA MELANOGASTER

By C. B. Bridges and T. H. Morgan



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THE THIRD-CHROMOSOME GROUP OF MUTANT CHARACTERS OF DROSOPHILA MELANOGASTER.

By C. B. Bridges and T. H. Morgan.

I. INTRODUCTION.

Since the work on *Drosophila* began in 1910, about 400 mutants have been found. The sex-linked characters, i. e., those due to genes in the first-chromosome, that were known to us in 1914, have been described in our paper, "Sex-linked Inheritance in *Drosophila*" (Carnegie Publication No. 237, 1916). The characters whose genes are in the "second-chromosome," that were known to us in 1915, have been described in our paper, "The Second-chromosome Group of Mutant Characters" (Part II, Carnegie Publication No. 278, 1919). Many other mutant characters in these two groups are now known to us, some of which have been described, but a greater number have been used in experiments dealing with special problems without having been described. It is our intention to publish details concerning these at some future time. The third group is here considered. In table 1, each of the third-chromosome mutants treated in this paper is listed in order of discovery, together with its symbol, its locus, and certain facts as to its origin and characteristics.

The mutants are dealt with in their chronological order, because each new mutant was first located with reference to the other characters then known. The information required to understand the successive experiments will, then, have been already furnished in the preceding sections. More accurate determinations of location in the case of several mutants have been made by using mutants that were discovered later, so that a certain amount of referring forward is unavoidable.

As a general rule, each mutant is treated as follows:

(1) The date of its discovery, and the circumstances under which it arose, are given, i. e., whether it appeared singly or in numbers, whether from a pair or in mass culture, etc. This information bears on the problem as to when, in the germ-track, the mutation occurred.

(2) A description of the character is given, its symbol, its range of fluctuation, and its availability for work, which last depends mainly on the certainty and ease of its separation from the wild-type. In many instances more than one part of the body is affected, and these different characteristics are also mentioned because they too are a part of the effect of the gene and are useful in identifying the mutant, in distinguishing it from its allelomorphs, and also from other mutants whose main effects involve the same part. Other characteristics, more obviously physiological, are also spoken of when they are known.

TABLE 1.—*Chronologically arranged list of third-chromosome mutants.*

Mutant gene.	Affects mainly—	See page	Symbol.	Locus.	Date found.	Found by—
					1910.	
With	Thorax-pattern	31	.....	p±	Jan. —	Morgan.
Olive-III	Body-color	36	ol-III	.....	Mar. —	Do.
Beaded	Wing-margin	37	Bd	93.8	May —	Do.
Pink	Eye-color	44	p	48.0	July —	Do.
Superwith	Thorax-pattern	35	.....	.....	Nov. —	Do.
					1911.	
Lethal-IIIa	Life	50	lIIIa	83±	Dec. —	Muller.
					1912.	
Ebony	Body-color	50	e	70.7	Feb. 15	Wallace.
Maroon	Eye-color	53	ma	50.7	Mar. 13	Bridges.
Lethal-IIIb	Life	64	lIIIb	p±7	Mar. —	Morgan.
White-ocelli	Ocellar-color	66	wo	76.2	June 21	Bridges.
Kidney	Eye-shape	72	k	64.0	June 26	Do.
Band	Thorax-pattern	79	bn	72.0	July —	Morgan.
					1913.	
Peach	Eye-color	82	p^p	48.0	Jan. 24	Bridges.
Truncate-Int.-III	Wing-shape	85	T-III	.....	Apr. —	Muller.
Sepia	Eye-color	86	se	26.0	May 10	Wallace.
CIII	III-crossing-over	89	CIII	75±	June —	Sturtevant.
Rough	Eye-texture	93	ro	91.1	June —	Muller.
Deformed	Eye-shape	93	Df	47.5	July —	E. Cattell.
White-head	Head-pattern	99	.....	.....	Aug. —	Morgan.
Sooty	Body-color	99	e^s	70.7	Oct. 20	Sturtevant.
Dwarf	Body-size	101	dw	51.0	Nov. 12	Bridges.
Spread	Wing-posture	105	sd	65±	Nov. —	Dexter.
Lethal-IIIc	Life	107	lIIIc	p+	Nov. —	Dexter.
Lethal-IIId	Life	107	lIIId	p±33	Dec. —	Liff.
Beaded-Int.-III	Wing-margin	108	.....	p—	Dec. —	Muller.
					1914.	
Spineless	Bristle-size	109	ss	58.5	Jan. 4	Bridges.
Cream-III	Eye-color	112	cr-III	36.5	Feb. 27	Do.
Port	Eye-color	125	.....	.....	Mar. —	Morgan.
Giant-III	Body-size	120	gt-III	64.0	Sept. 28	Bridges.
					1915.	
Safranin	Eye-color	126	.....	.....	Jan. 15	Do.
Dichæte	Wings, bristles	127	D	40.4	July 3	Do.
Tilt	Wing-posture	134	tt	40.2	Aug. 29	Do.
Bithorax	Body-segmentation	137	bx	58.7	Sept. 22	Do.
Smudge	Eye-texture	146	sm	15±	Nov. 5	Do.
Ski-III	Wing-curvature	149	si-III	46.5	Dec. 1	Clausen.
Dilute	Eye-color	151	.....	.....	Dec. 9	Bridges.
Curled	Wing-curvature	153	cu	46.0	Dec. 15	Morgan.
					1916.	
Two-bristles	Bristle-number	155	2b	58.3	Feb. 22	Bridges.
Black-leg	Leg-color	158	.....	p±	Feb. 23	Do.
Extra-scutellars-III	Scutellar-bristles	158	.....	59±	Feb. —	Payne.
Hairless	Bristles, hairs	161	H	69.5	Mar. 4	Bridges.
$Pink^3$	Eye-color	164	p^3	48.0	Mar. 21	Do.
Lethal-IIIe	Life	165	lIIIe	40.7	May —	Sturtevant.
Extended	Wing-posture	165	D^E	40.4	June 11	Do.
Lethal-IIIf	Life	168	lIIIf	39.1	June 30	Do.
Vortex-III	Thorax	168	vo-III	40.4±	Aug. 7	Bridges.

TABLE 1.—*Chronologically arranged list of third-chromosome mutants*—Continued.

Mutant gene.	Affects mainly—	See page	Symbol.	Locus.	Date found.	Found by—
Cluboid	Wings	169	.....	D±28	Sept. 15	Bridges.
D-extras	Bristles of D	132	.....	ro±	Sept. —	Sturtevant.
Ascute	Scutellum	170	as	43.5	Oct. 21	Bridges.
Scarlet	Eye-color	172	st	43.8	Nov. 18	Richards.
Intensifier-S	Star-eye	175	i-s	10±	Nov. 18	Bridges.
Benign-III	Tumor	179	be-III	25±	Nov. — 1917.	Stark.
Pink[4]	Eye-color	181	p^4	48.0	Jan. 7	Richards.
Ebony[3]	Body-color	181	e^3	70.7	Feb. 8	Sturtevant.
Divergent	Wing-posture	182	dv	24±	June 13	Bridges.
CHIP	III-crossing-over	183	CHIP	p−	July —	Payne.
Ebony[4]	Body-color	184	e^4	70.7	Sept. 27	Sturtevant.
Pale	Translocation of chromosomes	184	P	90±	Oct. 16 1918.	Bridges.
Semi-forked	Intensifier of f	187	.....	.....	Feb. —	Lancefield.
Glass	Eye-color and eye-texture	188	gl	63.1	Feb. —	Muller.
Rotated	Abdomen	190	.....	−	July 28	Bridges.
Mahogany	Eye-color	191	my	49.5	Nov. 13	Do.
Compressed	Eye-shape	193	cp	48.5	Nov. 27	Do.
Delta	Venation	197	Δ	66.2	Nov. 30	Do.
Hairy	Extra hairs	202	h	26.5	Dec. 11 1919.	Mohr.
Minute	Bristles	206	M	101.0	Feb. 8	Bridges.
Roughoid	Eye-texture	212	ru	0.0	Feb. 14	Sturtevant.
Cp-dilapidator	Cp-modifier	213	.....	68.5	Mar. 8	Bridges.
Lethal III-g	Life	214	lIIIg	h±12	Mar. 20	Do.
Port-b	Eye-color	214	.....	.....	Sept. 11	Do.
Warped	Wing	215	wp	52.0	Nov. 15	Do.
Cardinal	Eye-color	217	cd	75.7	Nov. 24	Johnson.
Claret	Eye-color	219	ca	100.7	Dec. 12	Bridges.
CHIM	III-crossing-over	220	CHIM	p+	Dec. 26	Do.
Bithorax-b	Body-segments	225	bx-b	59.5	Dec. 27 1920.	Do.
Roof-c	Wing-posture	228	.....	se−	Jan. 1	Bridges.
Dwarf-b	Body-size	228	dw-b	12±	Feb. 5	Do.
Minute-dIII	Bristle-size	231	MdIII	95±	Feb. 25	Do.
Rough[2]	Eye-texture	235	ro^2	91.1	Mar. —	Muller.
Delta-b	Venation	235	Δ-b	.....	Aug. 13	Bridges.
Dark	Eye-color	235	dk	.....	Sept. —	Clausen.
Minute-f	Bristle-size	236	Mf	105±	Sept. 9	Bridges.
Minute-g	Bristle-size	236	Mg	106.2	Sept. 27	Do.
Varnished	Eye-texture	237	vr	44.0	Oct. 22 1921.	Mohr.
Pointed-wing	Venation	238	Pw	94.1	Mar. 29	Bridges.
Shrunken	Body-size	241	wz	47.8	June —	Do.
Lethal-IIIh	Life	242	lIIIh	D−18	Nov. 28	Reed.
Minute-h	Bristle-size	244	Mh	.....	Dec. 20 1922.	Do.
Stripe	Thorax-pattern	244	sr	62.0	Feb. 6	Bridges.
Crumpled	Wings	247	cm	93.0	Mar. 2	Do.
Lethal-III	Life	249	lIII	63±	July 5	Do.

TABLE 2.—*Mean standard recombination per cents, Chromosome-III.*

Loci (alphabetical).	Primary data.		Secondary data.		Total data.	
	Per cent.	Numbers.	Per cent.	Numbers.	Per cent.	Numbers.
bx e	11.3	3,101			11.3	3,101
bx gl	4.1	4,468			4.1	4,468
bx H	13.6	819			13.6	819
bx ro			30.9	3,101	30.9	3,101
bx-b e			10.3	1,698	10.3	1,698
bx-b H	10.7	2,985			10.7	2,985
bx-b ro			28.9	1,698	28.9	1,698
cp Δ			18.0	310	18.0	310
cp ss	8.5	769			8.5	769
cp wo			23.0	769	23.0	769
ca M	0.3	1,842			0.3	1,842
ca Mg	5.5	2,041			5.5	2,041
cm-ca	6.7	658			6.7	658
Df ma	3.2	529			3.2	529
Df p	0.5	1,331			0.5	1,331
Df ss			7.3	317	7.3	317
Δ bn	6.8	281			6.8	281
Δ cd			10.0	1,012	10.0	1,012
Δ e	4.4	861	4.6	1,225	4.5	2,086
Δ H	2.9	4,469			2.9	4,469
Δ wo	10.3	310			10.3	310
D bn			26.8	1,974	26.8	1,974
D cd			27.1	616	27.1	616
D cbd	28.0	143			28.0	143
D cp	7.4	1,277			7.4	1,277
D cm			45.1	380	45.1	380
D Df	7.6	1,159	10.6	426	8.4	1,585
D Δ	21.9	645	20.9	310	21.6	955
D e	24.7	3,328	23.8	3,801	24.2	7,129
D gl	16.6	3,739			16.6	3,739
D H	23.1	9,221	23.7	7,802	23.4	17,023
D lme	0.3	913			0.3	913
D ma	10.7	716			10.7	716
D M			43.3	3,328	43.3	3,328
D p	6.5	7,786	8.1	1,607	7.0	9,393
D Pw	44.1	34			44.1	34
D ro	41.2	245	35.8	6,870	36.0	7,115
D st	3.4	6,280			3.4	6,280
D si	5.7	8,284			5.7	8,284
D ss	13.7	3,030	15.3	9,143	14.9	12,173
D sr	18.4	945			18.4	945
D ro-III	0.0	739			0.0	739
D wz	5.4	617			5.4	617
D wo			27.5	769	27.5	769
dw-H	32.5	1,527			32.5	1,527
dw-b D	25.8	124			25.8	124
dw-b H			37.9	124	37.9	124

TABLE 2.—*Mean standard recombination per cents, Chromosome-III*—Continued.

Loci (alphabetical).	Primary data.		Secondary data.		Total data.	
	Per cent.	Numbers.	Per cent.	Numbers.	Per cent.	Numbers.
e-cd	5.4	728			5.4	728
e-ca			29.5	1,289	29.5	1,289
e-M			25.9	6,013	25.9	6,013
e-Pw	21.1	199	23.7	2,257	23.5	2,456
e-ro	20.0	28,790			20.0	28,790
gt-wo	15.8	63			15.8	63
gl-Δ	3.6	4,471			3.6	4,471
gl-H	6.7	3,739			6.7	3,739
H-bn	2.4	2,370			2.4	2,370
H-cd	6.2	1,628			6.2	1,628
H-ca	31.5	3,883			31.5	3,883
H-cm	29.5	380			29.5	380
H-e	1.2	3,992			1.2	3,992
H-M			32.5	1,842	32.5	1,842
H-Mdiii	24.0	362			24.0	362
H-Mg			34.1	2,041	34.1	2,041
H-ro			21.9	4,877	21.9	4,877
H-wo	6.8	6,144			6.8	6,144
h-Df			27.2	305	27.2	305
h-D	14.2	8,253			14.2	8,253
h-e	36.6	1,087			36.6	1,087
h-liiig	11.7	257			11.7	257
h-liiii	25.7	391			25.7	391
i-s-D			28.3	590	28.3	590
i-s-h	24.3	590			24.3	590
k-bn	8.4	237			8.4	237
k-e	8.1	2,104			8.1	2,104
liiif-D	1.3	78			1.3	78
liiih-D	17.7	96			17.7	96
liiih-H	48.9	258			48.9	258
ma-bn	20.8	235			20.8	235
p-bn	18.1	237	22.8	2,503	22.4	2,740
p-Bd	40.0	360			40.0	360
p-Δ	18.9	281			18.9	281
p-e	23.7	4,738	19.5	626	23.2	5,364
p-H	21.2	3,423	20.2	243	21.1	3,666
p-k	13.8	2,015	18.5	383	14.6	2,398
p-liiib	7.0	525			7.0	525
p-liiid	32.7	900			32.7	900
p-lhd	7.7	70			7.7	70
p-ro	34.0	626			34.0	626
p-ss	10.4	3,445			10.4	3,445
Pw-ca	7.6	1,289			7.6	1,289
ro-ca	11.6	1,889	9.4	1,748	10.6	3,637
ro-cm	2.0	658			2.0	658
ro-M	9.0	6,013			9.0	6,013
ro-Pw	3.3	2,257			3.3	2,257

TABLE 2.—*Mean standard recombination per cents, Chromosome-III*—Continued.

Loci (alphabetical).	Primary data.		Secondary data.		Total data.	
	Per cent.	Numbers.	Per cent.	Numbers.	Per cent.	Numbers.
ru-D	35.9	192	38.9	1,389	38.5	1,581
ru-e			46.3	2,241	46.3	2,241
ru-H			45.2	980	45.2	980
ru-M	45.8	856			45.8	856
ru-p			41.8	2,241	41.8	2,241
ru-st	39.9	2,241			39.9	2,241
ru-se	25.7	6,364			25.7	6,364
st-Df	6.5	902			6.5	902
st-e			28.0	2,241	28.0	2,241
st-H	25.4	552			25.4	552
st-p	4.7	7,224			4.7	7,224
st-ss			14.2	1,615	14.2	1,615
se-bx	29.2	3,920			29.2	3,920
se-D	13.8	10,454			13.8	10,454
se-e	36.9	2,685	33.2	23,060	33.6	25,745
se-H			33.7	5,945	33.7	5,945
se-h	0.5	1,439			0.5	1,439
se-M			47.1	6,013	47.1	6,013
se-Md			49.8	263	49.8	263
se-Mf			52.4	229	52.4	229
se-ro			42.8	24,639	42.8	24,639
se-ss	26.8	13,713	24.9	3,030	26.5	16,842
se-wp	23.9	1,898			23.9	1,898
se-wz	45.4	229	37.8	1,898	37.8	2,127
si-ss	11.4	7,505			11.4	7,505
ss-bx	0.2	8,853			0.2	8,853
ss-Δ	7.9	1,171	8.6	5,858	8.5	7,029
ss-e	12.0	19,606	11.4	861	12.0	20,467
ss-gt	6.8	88			6.8	88
ss-gl	4.9	4,471	4.4	4,468	4.6	8,939
ss-H	9.9	4,489	11.1	1,387	10.2	5,876
ss-k	7.7	1,226			7.7	1,226
ss-ro	24.3	185	30.9	18,899	30.9	19,084
ss-sr	3.5	3,054			3.5	3,054
ss-wo	13.9	459	16.8	4,556	16.5	5,015
sr-Δ	5.5	1,387			5.5	1,387
sr-H			7.7	1,387	7.7	1,387
tt-D	0.2	530			0.2	530
tt-p	7.8	665	6.2	530	7.1	1,195
2b-ss	0.2	1,980			0.2	1,980
wp-H	19.7	1,898			19.7	1,898
wp-wo			26.5	1,898	26.5	1,898
wz-p	0.2	617			0.2	617
wo-Mf	31.5	229			31.5	229
wo-ro	15.8	3,179			15.8	3,179

(3) It is stated whether the character is dominant, recessive, or intermediate.

(4) The evidence is given for referring the mutant to a particular linkage group, or to more than one group in case of multiple gene mutants.

(5) The linkage experiments, on the basis of which the locus within the chromosome has been determined, are also reported.

(6) When a mutant has been found more than once the circumstances of its rediscovery are given.

(7) If any allelomorphs are known they are described and their characteristics contrasted with the first-discovered gene.

(8) Any other peculiarities, such as specific interaction with other mutant characters, intensification effects, mimicry, and masking effect on other characters are reported.

(9) The "evaluation" of the character is given, i. e., a consideration of the mutant from the standpoint of its usefulness, taking into account many of the points mentioned above.

NOMENCLATURE AND SYMBOLS.

In the main we have used the same system of symbols as in the preceding studies. When a new mutant is found, it is given a name that hits off its most striking departure from the wild-type; for example, peach (eye-color), ebony (body-color), spread (wings), curled (wings), spineless, hairy, etc. Many of the mutants differ strikingly in more than one characteristic from the wild-type. In such cases the mutant is usually named for the most striking of its peculiarities; for example, "Delta" was named on account of the delta-like expansion where the longitudinal veins join the marginal vein, although the same mutant is characterized by small, rough eyes, by a bristle modification, and by several other slight differences.

When the development of a mutant character is dependent upon more than one gene, the genes involved receive the same name, such as "olive," and one (or both) of the genes is further designated by the addition of Roman numerals for its chromosome, as olive–II, olive–III; vortex–II, vortex–III; Ski–II, ski–III; and giant–III, giant–II.

In cases where mutants look alike (so called mimics) we have named the one found later the same as the one first found but with a distinguishing letter, thus, safranin (III), safranin–b (II); roof (I), roof–b (II), roof–c (III); dwarf, dwarf–b; bithorax (III), bithorax–b (III). A mixed procedure was used in naming the creams; for example—cream, cream–a, cream–b, cream–c; but also cream–II and cream–III, the Roman numerals in this case designating chromosome but not interdependence.

In the case of a lethal mutant, we usually have no other way of characterizing it than that the gene kills, how or when not being known in most cases. In naming the lethal we have generally followed a numeral system, such as l-1, l-2, l-7, etc., for those in the X-chromosome in the order of discovery; and for those in the II– and III–chromosomes lIIa, lIIb, lIIc, and lIIIa, lIIIb, lIIIc, etc.

There is a mutant type showing small bristles that frequently appears (Minute). It is dominant. Its hatching is delayed. It is lethal when homo-

zygous. After we became aware of this kind of mutant, about 35 were picked up during the first year. They were numbered in the order of their appearance, thus, Minute (M), Minute–2 (M2), Minute–3 (M3), etc. It was soon found that a majority of these "Minutes" were identical, and were due to the absence of a IVth–chromosome. These were renamed "Diminished." The rest were found to be members of the II– or III–linkage groups, and were distinguished as a, b, c, in the order of their occurrence. Thus, Minute–2 became Minute–b (II), Minute–18 (III) became Minute–f, and Minute–23 (III) became Minute–g. In one case, where the appearance of a Minute depended on the presence of two genes, one in the second and one in the third linkage-group, the genes were called Minute–d–II and Minute–d–III.

In cases of multiple allelomorphs, two systems have been followed: The earlier plan was to give each allelomorph a descriptive name, such as pink and peach, ebony and sooty; but in cases where distinguishing names are more difficult to apply, or where there are too many allelomorphs to find names for, we have resorted to numbering; thus, a third allelomorph of pink was called pink^3 and a fourth, pink^4. Likewise the third allelomorph or ebony was called ebony^3 (which was a lethal), and the fourth, ebony^4, etc. In the case of the sex-linked mutant cut, which has appeared as often as 14 times, the number given to the mutant (ct^2, ct^3, ct^{13}) applies to the order of appearance. Some of the cuts have probably been due to the reoccurrence of the first cut (or at least to a mutant change indistinguishable from it); but in general an attempt will be made to give numbers only to those allelomorphs that are distinguishable.

In order to distinguish dominant mutants from recessive mutants, we capitalize the first letter of the name of the dominant and use these initial capitals for the symbols, as Dichæte (D), Star (S), Bar (B).

In general, the symbol of any mutant is the initial letter of the name, which is a small letter for a recessive; thus, p=pink, and a capital letter for a dominant; thus, D=Dichæte. Since we have several mutants beginning with the same letter (such as spineless and sepia) it is often necessary to use the first letter and another letter that suggests the name of the mutant: thus, ss=spineless, bx=bithorax, etc. Formerly the second letter was written as a subletter; thus, b_x, but this caused so much trouble to the printer and to the proof-reader that we now write the second letter on the same line as the first (bx).

The wild-type fly, not showing any mutant character, is designated by +, which is to be read "wild-type" or "normal," and also "standard" when the wild-type is unknown. The symbol is also used to designate the wild-type allelomorph of any mutant character. Thus, (p=pink) and the wild-type eye-color, red, is indicated by +; i. e., + stands here for the allelomorph of pink. The symbol $\frac{D}{+}-\frac{+}{p}$ stands for a Dichæte fly that has come from a cross of dominant Dichæte to recessive pink, i. e., it has Dichæte (D) and the wild-type allelomorph of pink (+) in one chromosome, and in the other the wild-type allelomorph of Dichæte (+) and the recessive pink gene (p). In case the use of + for the wild-type gene or character is not specific enough for the purpose in mind, an exponent can be added to designate the particular wild-type; thus, $+^p$=the wild-type allelomorph of pink or the not-pink character,

and $+^{D}$ = the wild-type allelomorph of Dichæte or the not-Dichæte character. An alternative scheme sometimes used in this laboratory is to precede the sign for the mutant by $\neq$ (not-equal-to) to designate the wild-type allelomorph or character; thus, $\neq$ D is read "not-Dichæte," or the wild-type allelomorph of Dichæte, and $\neq$ p is read "not-pink."

We no longer prime the symbols used in connection with the dominant mutants; thus, H, not H′, is the symbol for Hairless.

In the case of multiple allelomorphs we make use of exponents. The initial letter of the first member of the series (such as w for white, p for pink, e for ebony) is used throughout the series, and to this an exponent is added to indicate the particular allelomorph (thus, w^{e} = eosin, p^{p} = peach, e^{s} = sooty). Where the multiple allelomorphs are numbered rather than named, a number is used as the exponent; thus, p^{3} and p^{4}, e^{3} and e^{4}.

The symbol for the mutant stands equally for the character, the gene, or the locus. The context tells which is intended.

In the case of *Drosophila,* where the wild-type is known, we give, as stated, a special name to each *mutant* type, whether it is dominant or recessive; but in forms where the wild-type is unknown (or not certainly known) the same system can be followed. In such a case we may usually take the less common form as the mutant; and, if it is a recessive, use its initial small letter as the symbol of the mutant allelomorph.

When a fly shows two or more mutant characters that are from different linkage groups, we find it convenient in practice to write first those of group I, then those of group II, then those of group III, etc.; thus, eosin Star Dichæte. When the mutant characters are in the same group, the names are written in the order of their position with respect to the zero (or left end of the map); thus, eosin vermilion forked, and roughoid hairy scarlet spineless.

RELATION BETWEEN RECOMBINATION AND CROSSING-OVER.

When linked genes enter a cross certain individuals in the second generation may show new combinations of the characters that entered from the different parents. Thus, if Dichæte is crossed to pink, and the F_1 female is back-crossed to a pink male, most of the flies are of the two original types, Dichæte or pink; but a small number of the offspring are both Dichæte and pink or neither (i. e., wild-type). These two latter classes are called "recombination classes" and the "percentage of recombination" may be found by dividing the sum of the recombination classes by the total number, and multiplying this decimal fraction by 100. (Example: P_1 mating, Dichæte × pink; B. C. mating, F_1D ♀ × p♂; B. C. offspring: original combinations, D = 347, p = 339; recombinations, D p = 25, + = 29; sum of recombinations = 54; total individuals = 740; percentage of recombination = $\frac{54 \times 100}{740} = 7.3$.) The use of the term "recombination" in this technical sense is a shortening of the full term "recombination of linked characters."

When three (or more) mutant characters are involved in a linkage experiment, the percentage of recombination is calculated separately for two loci at a time, as though no other characters were present. In a back-cross experiment where three loci are followed, such as that of Dichæte Hairless × claret

(table 170, p. 219), eight classes are expected. Two of these, D H and ca, are the original combinations (O recombination). Two others, D ca and H, are recombinations that result from crossing-over between the loci for Dichæte and Hairless (recombinations for crossing-over section No. 1). Two other "parallel" classes, D H ca and +, are simple recombinations for crossing-over section No. 2. Finally, the two parallel classes D and H ca are "double recombinations" representing crossing-over in both sections simultaneously (1–2 double recombinations).

Two characters show linkage if their genes are carried by the same pair of chromosomes. We may suppose that chromosomes are like strings of beads, or chains, and that the genes correspond to beads, or to links. The two chromosomes of a pair come to lie side by side with allelomorphic genes at the same levels. Crossing-over is the breaking across at a given level (between genes) of the two chains, and the union of the parts in an interchanged relation. The section of one homologue lying to the left of the breaking-point becomes joined to the section of the other homologue lying to the right of the breaking point.

If such a crossing-over should occur between the levels occupied by the genes Dichæte and pink, then individuals showing recombination of these characters would result. If the locus of pink is close to that of Dichæte, then only rarely will such a crossing-over point fall between them, and the percentage of recombination individuals will be low. If the locus of a third mutant, viz., Hairless, lies farther along the chromosome than does pink, the number of crossings-over between D and H should be greater than that between D and p, for the D–H crossing-over includes all the D–p and also whatever occurs between p and H. We may make a map of the locations of the genes along the chromosome, using as our unit a length of chromosome such that there is one case of crossing-over per average hundred gametes. If we map two loci at 15 units distance from each other, that means that each 100 gametes include 15 cases in which crossing-over had occurred between the levels occupied by these two loci. Some gametes may represent 2 or 3 or more cases of crossing-over, so that the number of cases of crossing-over may exceed the number of gametes. Thus (see fig. 1) there are 106.2 cases of crossing-over between roughoid and Minute–g per 100 gametes. But if two crossings-over occur in the section between two given loci, the one neutralizes the other, and no recombination is apparent. That is, whenever two loci are so far apart that some double crossing-over takes place between them, twice that amount of crossing-over fails to be represented by recombination. The map-distance (total number of cases of crossing-over per 100 gametes) is only equal to the percentage of recombination when the loci are so close together that the number of cases of double crossing-over between them is negligibly small. The practical equivalence of recombination with crossing-over holds for distances under 5 units in the middle of the third chromosome, and for distances up to 15 units in some other regions. With distances greater than these, the number of cases of crossing-over that do not give rise to recombination becomes increasingly greater. The percentage of recombination has not been observed to rise beyond 50, although the number of cases of crossing-over may rise above 106. We can demonstrate that such double crossing-over is occurring, and find out how much is occurring, by following other loci situated between the two in question. Thus, a back-cross in which the additional loci

scarlet pink spineless and glass between Dichæte and Hairless are used will tell how many cases of crossing-over would be unrepresented if only Dichæte and Hairless had been followed. This is found to be 3.4 per 100 gametes (see fig. 1). Dichæte and Hairless give a standard recombination per cent of 25.7, and accordingly the map-distance between Dichæte and Hairless is 25.7 + 3.4 = 29.1. By studying a large range and variety of experiments in which intermediate points were followed, we can find what map-distance corresponds to each recombination per cent and vice versa. In figure 1 each curve represents by its height above a given point the number of cases per 100 gametes by which recombination is less than map-distance. These curves are different for different regions of the chromosome. The curve originating at hairy cuts the ordinate at Dichæte at a height of 0.5 unit, the ordinate at spineless at 4.8 units, at rough at 19.4 units, etc. Likewise, the ordinate at rough cuts the curve originating at spineless at a height of 1.5 units, that originating at pink at a height of 4.8 units, that from Dichæte at 9.2 units, etc.

In the curves of figure 2 this same relation is expressed differently. The curve originating at a given point shows by the height of the ordinate at each other point the standard percentage of recombination. Thus, hairy gives 19.8 per cent of recombination with pink, 27.2 with spineless, 34.3 with ebony, and 45.3 with rough, while the corresponding map distances are 21.5, 32.0, 44.2, and 64.9 respectively, as shown by the height at which the straight line having its origin at hairy cuts these same ordinates.

The application of the knowledge of the correspondence between recombination and map-distance to the construction of maps will be considered in more detail in another section.

METHODS FOR DETERMINING TO WHICH LINKAGE GROUP A MUTANT BELONGS.

As the number of mutants increased it was found that some of them did not show independent assortment (Mendel's second law), but were "linked" to other characters. In time the four linkage groups of *Drosophila* emerged.

A mutant is a member of the first group if it shows sex-linked inheritance and shows linkage to previously known members of this group. A mutant is a member of the second group if it shows linkage to black or to some other mutant already known to be linked to black. Similarly, a mutant that shows linkage to pink is a member of group III.

Owing to the fact that there is no crossing-over in the male, it is easy to determine to which group an autosomal mutant belongs. For example, if a new not-sex-linked recessive comes up, it will be found to give in F_2 a 2 : 1 : 1 : 0 ratio with any other recessive of the group in which it lies. Thus, the new mutant spineless crossed to pink gave in F_2, 2 wild-type: 1 pink: 1 spineless: 0 pink spineless, a ratio which showed that spineless is linked to pink. On the other hand, when spineless was crossed to a mutant in the second group, namely, black, there was found in F_2 the ratio 9 wild-type: 3 black: 3 spineless: 1 black spineless—in other words, a ratio indicating free assortment, which means that spineless does not belong to linkage group II.

A more advantageous method, that is now more generally used, consists in crossing a new recessive mutant to a fly that has both the II–chromosome

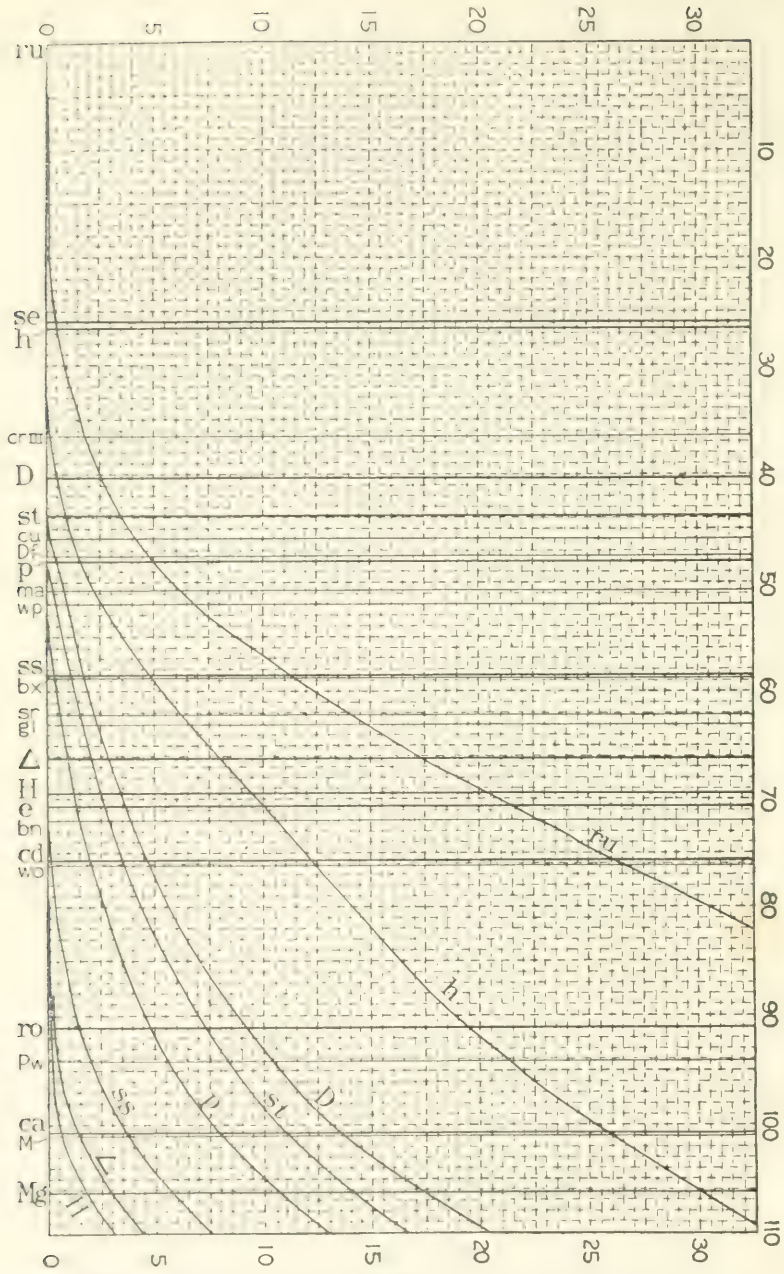


FIG. 1.—Line-map and correction curves for chromosome-III.

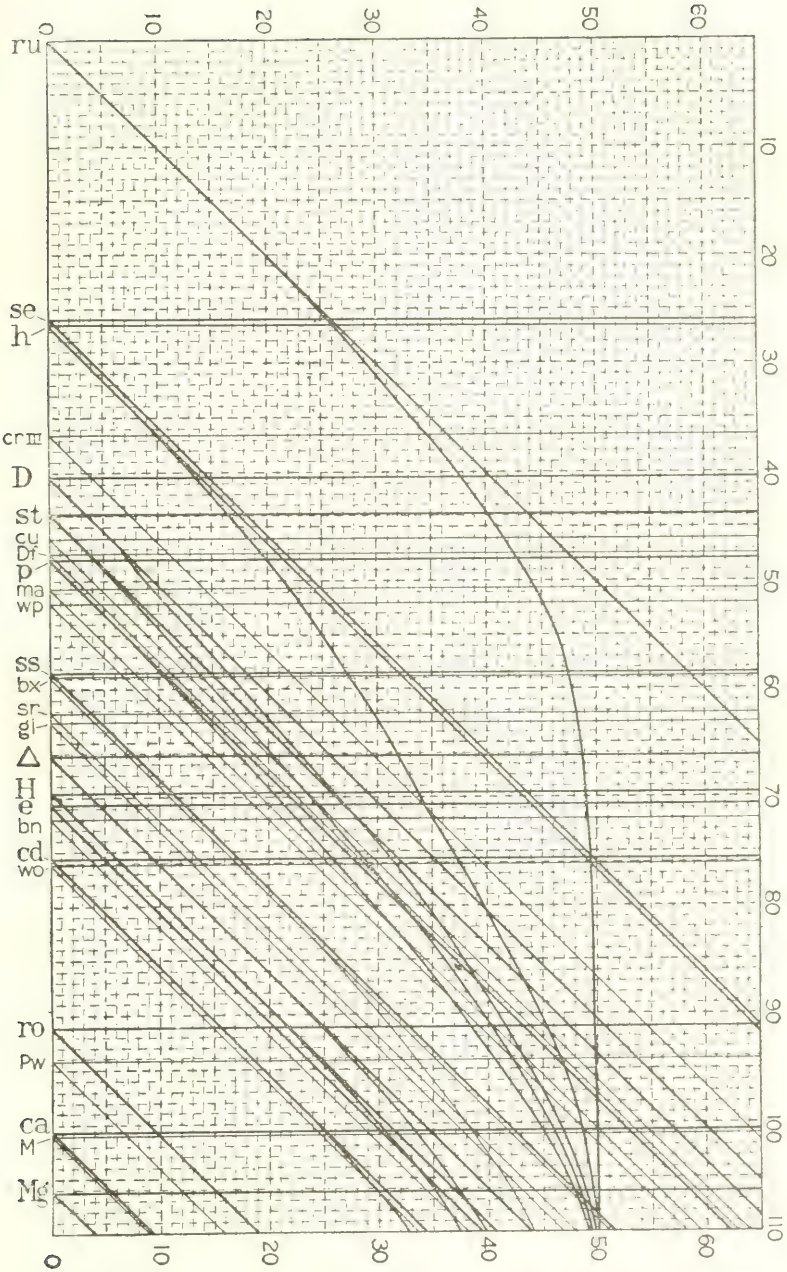


FIG. 2.—Line-map and recombination curves for chromosome-III.

dominant Star and the III–chromosome dominant Dichæte. The F_1 Star Dichæte male is then crossed to a female of the new mutant (or to a wild-type F_1 female that is heterozygous for the mutant). If the new mutant is in the III-chromosome, none of the Dichætes that occur in the back-cross offspring will show the new character, while, on the other hand, half (or a quarter) of the Star flies will show it. If the new mutant is in the II–chromosome, none of the Star flies will show the new character, while half (or a quarter) of the Dichæte flies will show it.

METHODS FOR FINDING THE POSITION OF THE LOCUS OF A MUTANT WITHIN A CHROMOSOME.

The advantage of the above procedure is that when the linkage group has thus been found, one can proceed at once with the tests of the location of the new gene within its linkage group. For instance, after such a spineless by Star Dichæte male back-cross test, Dichæte females heterozygous for spineless are mated to spineless males (female back-cross). The progeny will show that there is about 16 per cent of recombination for Dichæte and spineless. The Star can be easily eliminated from the further experiments, because it is dominant and can be seen whenever present.

One other determination is required before the locus of spineless can be known to be to the right or to the left of Dichæte; its relation to some other known locus in this chromosome must be determined. The dominant Hairless is generally used in such cases. If the new recessive is crossed to the double dominant Dichæte Hairless, and the F_1 Dichæte Hairless female is crossed to a male of the new mutant stock, the progeny show the recombination percentage of the new mutant and Hairless, giving the other determination required; and also, since this is a three-point back-cross, the relative position of the new gene is shown by the double recombination classes, which are the smallest of the classes produced.

The tests for the location of the new recessive mutant hairy illustrates the tests already described and some further methods. Thus, a hairy × Star Dichæte male back-cross test had showed that hairy is in the third chromosome (see table 146, p. 203). A female back-cross test, viz., hairy by Dichæte (see table 147), showed that there is about 16 per cent of recombination for hairy and Dichæte. The locus of hairy was thus shown to lie about 16 units to the left or to the right of that of Dichæte. A three-point back-cross of hairy to Dichæte Hairless (see table 148) gave 30 per cent of recombination for hairy and Hairless, showing that hairy is to the left of Dichæte, for Dichæte with Hairless gave only 22 per cent recombination. The order of the genes, hairy Dichæte Hairless, was shown also by the fact that the smallest classes (double recombinations) were hairy Dichæte and Hairless. The locus of hairy was thus roughly placed at 16 units to the left of Dichæte, or near sepia.

The next point was to find out on which side of sepia hairy lies. A comparison of the hairy Dichæte with the sepia Dichæte recombination per cents should show which is farthest removed from Dichæte; but in this case the values were so similar that the abnormally large variation within each made either order possible. In such a case, it is necessary to secure the double recessive sepia hairy (or hairy sepia) and use this in a three-point cross with

some other mutant, preferably Dichæte. An attempt to get this double recessive failed, although carried out on a large scale, which was a strong indication that the loci are very close together.

Another method had to be resorted to in order to find out the relative position of hairy with respect to sepia. This is the method of parallel back-crosses, the purpose of which is to determine the distances of hairy and of sepia from a common locus (here Dichæte) under conditions as nearly identical as possible. The method is as follows: hairy Dichæte is first crossed to sepia; some of the F_1 females (A) are back-crossed to hairy; others (their sisters) are bred to sepia (B). From lot A we get a recombination per cent for hairy and Dichæte, and from lot B for sepia and Dichæte. These two values are comparable, since the constitutions of the two lots of mothers are the same. This holds even if crossing-over modifiers were present in the parental stocks, provided enough F_1 females are used in A and B. Such parallel back-crosses gave a value of 13.5 for sepia Dichæte (table 150), and for hairy Dichæte a value of 12.5 (table 149). It follows that the more probable order of the genes is sepia, hairy, Dichæte. But when dealing with differences as small as these, very large numbers of individuals would be necessary to establish the relation. In the present case this probable order of sepia hairy was made the basis of an attempt to secure a sepia hairy double recessive. Thus, some of the (back-cross) sepia Dichæte individuals from B (table 150) should have resulted from crossing-over between sepia and hairy. One of the III–chromosomes in such a fly is sepia hairy Dichæte (and its mate sepia). Two such flies mated together should give sepia hairy Dichæte offspring. But if sepia and hairy are very near together, there is a very small chance for any particular sepia Dichæte fly to be of the above constitution, and less still that any two mated should both be right, so that only from very large numbers of such matings would the double recessive be obtained. An improvement of the method is as follows: Some recombination sepia Dichæte flies are crossed to hairy. If any of the offspring are hairy, these have come from a sepia hairy Dichæte cross-over chromosome. Further matings of these hairy offspring will give sepia hairy stock. The actual experiment gave this result, which proved that the order sepia hairy is correct. If the parallel back-crosses had indicated that the order of the loci is hairy sepia Dichæte, then to secure the double recessive for the back-crossing, the hairy not-Dichææte from A (table 149) would be mated to sepia, and if any sepia offspring were produced they must have originated by crossing-over between hairy and sepia and would give a hairy sepia stock after inbreeding.

Having obtained sepia hairy (the double recessive), one is in a position to determine the distance between sepia and hairy by a direct back-cross. Thus, sepia hairy was mated to Dichæte and the F_1 females were back-crossed by sepia hairy (table 151). The recombination classes due to crossing-over between sepia and hairy were sepia Dichæte and hairy, which together constituted 0.5 per cent of the offspring. This corresponds to half a unit of distance between the loci.

In the preceding experiment the mating might better have been made sepia Dichæte $\times$ hairy, since this is the best of the four possible ways in which these three genes can enter the back-cross. The recombination classes of the offspring that result from the above back-cross are more equally viable than when

the F_2 genes enter in any other combination. Practically all the flies should have one or two mutant characters and almost none have all three or lack all three. To secure the maximum evenness in viability, in a three-point back-cross, the genes for the two mutants farthest apart enter from one parent and for the one lying between from the other parent. When more than three loci are involved, the mutant genes should lie *alternately;* thus, as shown in the diagram $\frac{rn \quad D \quad p \quad e}{h \quad st \quad ss}$, the seven genes alternate between the two members of the same pair of chromosomes.

Having first obtained approximately the position of the new mutant locus with respect to two known loci in its group, preferably with respect to some two whose positions are among those best known, and, secondly, having found the position of the new gene with respect to the best known of the mutants in its immediate neighborhood, we proceed, thirdly, to find its relation to less accurately placed genes in the same neighborhood.

In order to do this, double recessives must first be obtained, and in this process the serial order will be discovered. The double recessives are then used in connection with some other well-established locus in a three-point back-cross. The results furnish evidence as to the relative distances between the points involved.

When several genes are near together, as in the neighborhood of pink, the finding of the serial order and of the relative distances involves many such experiments. This determination is so laborious that, as a rule, it is carried out to completion only in the case of the most useful mutants.

RELATIVE USEFULNESS OF MUTANTS IN CROSSING-OVER STUDIES.

The mutants that are most used as a basis of reference are those: (1) most easily and surely separable from the wild-type, (2) not markedly inferior to the wild-type in viability or in fertility and productivity, (3) which do not interfere with the classification of other reliable mutants (4) whose position in the chromosome is most accurately known with reference to each other, and (5) which occupy strategic points, namely, the ends of the chromosomes, the middle, and other points evenly distributed along the chromosome, just near enough together so that double crossing-over is negligible, but not so near together that single crossing-over is too infrequent. Mutants that fulfill these qualifications are mutants of the first rank. Failure in some of these requirements (such as being too close to some other first-rank mutant) relegates them to the second rank, etc., down to the poorer mutants that are almost worthless (see fig. 3).

SOME CONSIDERATIONS UPON THE RELATIVE VALUE OF CROSSING-OVER DATA.

It is necessary to explain fully the methods by which accurate and comparable data are obtained, because experience has shown that the significance of the facts has not been grasped; and data from different sources have been used by several writers who have attempted to point out seeming contradictions in our conclusions or to bolster up new views that are untenable when all the evidence is taken into consideration.

The only crossing-over data known to be strictly homogeneous are those derived from a given single pair, and from their progeny for only a very short period of egg-laying at a constant temperature. The time-limitation follows from the fact that the freedom of crossing-over is known to change with the age of the female. The amount of this change is different for different chromosomes and for different parts of the same chromosome. It is nearly negligible for the X–chromosome, and for long sections of the second and of the third. The change is particularly great for the mid-region of the second-chromosome (about purple) and of the third (about pink). But experiments have shown that there is enough uniformity from female to female in the course of this age-change so that if some standard period of egg-laying is adopted the different lots of data are workably uniform even for sensitive regions. The usual period of egg-laying allowed is 10 days, which is the average time from the mating of a pair to the emergence of their first offspring if the temperature is constant at 24° to 25°. If the temperature is varying, or is constantly higher or lower than this, it is probable that the period from mating of parents to emergence of offspring will give a more nearly constant condition than would a ten-day period. Most of the work of late years has conformed to the mating-to-emergence standard.

The temperature-limitation is just as important as the age, for the same regions that are particularly sensitive to change with age are also particularly susceptible to change with temperature. A temperature of 24° to 25° has been adopted as standard.

Besides the variations in crossing-over due to these, and probably to other environmental factors, which can be made nearly constant or random, it is known that there are a large number of mutant conditions that decrease or, more rarely, increase the amount of crossing-over for particular regions, for a whole chromosome, or for all the chromosomes. Some of these variations are very striking, and all data influenced by them differ characteristically from standard data, and can be separated out and made the bases of distinct systems of crossing-over with specific maps. Others of these variations are slight in extent or are rather widespread, and thus the selection of standard data is difficult and uncertain.

There are several considerations in planning experiments that aid in the judgment as to what data are homogeneous or standard. The region in the third chromosome that is observed to be most variable is that from sepia to spineless or sometimes to ebony. Of this region the most sensitive section seems to be that between scarlet and pink.

To be comparable, then, data must be uniform with respect to this linkage region. Experiments should be planned so as to include a means of observing how much crossing-over is occurring in at least part of this sensitive region. The use of Dichæte, pink, and spineless in connection with the other loci used is perhaps the most practical check. The simultaneous use of hairy, Dichæte, scarlet, pink, spineless and ebony is the best check at our disposal at present. Of these the most easily dispensed with is ebony, the next scarlet, and the next hairy. The more loci followed, the surer is the analysis of the data.

A second consideration is that the section covered by an experiment should have a first-rank locus at each end. An experiment should span a section that is well known and be anchored at the ends to thoroughly established loci.

A two-point experiment of which one point is an unknown ties on at only one end. In such a case there is no means of determining to what system of linkage the value obtained belongs—whether it is a standard value or one modified. A three-point experiment of which one point is the unknown and two are first-rank loci gives a means of judging whether the crossing-over is standard or not. The two knowns should not be more than 30 units apart and should be in the same region with the unknown. The most favorable type of experiment flanks the unknown on each side by the closest first-rank mutant, and if these two do not furnish a check over the sensitive region, such a check should be added by use of one or more additional loci in that region.

Unless stocks have been especially made homozygous, and tested as to their constitution, they are liable not to be homogeneous for crossing-over. This situation is best met by using exclusively pairs for the P_1 matings, and then using enough cultures in the next generation from the pair selected to make heterogeneity readily detectable. The most frequent case of heterogeneity would be that in which one parent was heterozygous for a modifier. Eight back-cross culturs from the F_1 daughters of a P_1 pair should give representatives of both systems and a check upon homogeneity.

The larger the number of individuals in an experiment the surer it is that the recombination per cents are characteristic of the crossing-over system underlying them, and the safer the judgment that this system is the standard or a modified system. But it is not advisable to go on indefinitely accumulating data in a particular experiment, for the closeness of approach to the underlying ratios is not in direct proportion to the increase in numbers. Ordinarily, the eight cultures that are enough to show whether the data belong to one system are enough to show what this system is. It is better to settle a point by several relatively small and independent experiments than by a single large one. A single experiment can demonstrate that a particular situation exists, but the generality of that system can only be established by finding it elsewhere.

SUCCESSIVE MAPS OF A GIVEN CHROMOSOME.

The map of a chromosome grows in completeness and accuracy step by step with the discovery of mutants and the accumulation of crossing-over data. Each stage forms the basis for the planning of new experiments, which in turn enable a new map to be calculated. The serial order of the loci is the most important information represented by the map. The location of a new mutant within the series of those already mapped is made by the methods already given (p. 14ff). The distances between loci are also represented, but this type of information is by no means so unquestionable as is the serial order. The serial order can be established absolutely, except in those cases where the loci of recessives are so close together that double recessives are too difficult to obtain. The accuracy of map-distances depends upon the quantity and homogeneity of the data. As new data accumulate it becomes advisable occasionally to discard the old map completely, as far as distances are concerned, and to calculate a new map, considering all the data simultaneously.

LISTING THE DATA.

The first step is to make a collection, the items of which are separate crossing-over experiments. When all the data to be considered have thus been listed, the whole is examined as to homogeneity. Certain experiments are known from their pedigree and their characteristics to involve Chi or some other striking and recognized crossing-over modification. These experiments are separated out, and all those of a given system considered together in constructing a map that corresponds to that system. The bulk of the data remains, and is now carefully examined for the character of the heterogeneity that may be still present. The first test applied is that of similarity of given recombination per cents.

EXAMINATION OF VARIABILITY.

The data of each experiment are calculated as to the recombination per cents for each two loci followed in it. Thus a two-point experiment (A, B) gives a single A–B recombination per cent of a particular value. Each three-point experiment (A, B, C) gives 3 recombination per cents—A–B, A–C, and B–C; each four-point experiment (A, B, C, D) gives 6 recombinations—A–B, A–C, A–D, B–C, B–D, C–D; each five-point gives 10, and so on. The recombinations for a given two loci (C, D) are observed in one or more two-point experiments, and perhaps in a dozen different multilocal experiments. These different sets of data are now assembled. All the C-D data are listed together for comparison. A curve is made of the distributions of the values. The curve is examined to see if it is bimodal or has an unusually wide range. Values that are strikingly outlying are examined as to pedigree and as to the values given by the other recombinations in that same experiment. Also, the number of individuals in each experiment is considered. Two experiments that involve many individuals may be significantly different with a rather small absolute difference, while two small experiments may differ widely without belonging to different systems. A calculation of the standard error ($\sqrt{Npq}$) can be easily made and it helps in this judgment. As stated previously, the data for the third chromosome showed considerable significant variability in the mid-region (h to ss) and little in the end-regions.

The next step is to consider how many systems are necessary to handle the above data adequately. The different experiments can be arranged in order of diagnostic value with considerable accuracy. The main factors in this weighting are: (1) the number of loci followed; (2) the closeness with which the variable region is checked; (3) the number of individuals involved; (4) the character of the mutants used in the experiment, i. e., whether inviable, subject to slight errors in classification, etc.; (5) the conditions under which an experiment was made and by whom. The judgment as to the number of systems needed must be based primarily upon only the most significant experiments; and the remaining experiments, several times as numerous, are then examined as to pedigree and characteristics and seen to be in conformity with one or another of the systems. In both steps the chi square (χ^2) test of homogeneity is of assistance.

It is to be remembered that in general slight mutations are much more frequent than larger ones, and likewise with crossing-over it is to be supposed

that most of the variability can be weeded out by the elimination of the few systems with the largest departures from standard. It is not ordinarily desirable to try to sift the data further than this, for the uncertainty of judgment becomes greater the slighter the difference, and what is needed is a kind of composite picture of the standard, together with the slight plus and minus deviations that are liable to be met with in ordinary experiments.

SEPARATION OF DATA INTO SYSTEMS.

It seems probable that the standard and three other systems are sufficient for the residual third-chromosome data, that is, the data remaining after the identifiable crossing-over variations, such as Chi, have been taken out. Plotting the values given by Dichæte and Hairless, and by other loci spanning this region, results in a bimodal curve with the cleft at about 26 per cent recombination. The larger values seem to depend on a high pink spineless and spineless ebony recombination per cent, i. e., to high crossing-over to the right of the midpoint. Another system is characterized by a low Dichæte scarlet value (about 1.4 instead of 3.4), and, in general, by low crossing-over to the left of the mid-point.

There are also a few experiments, in which hairy is involved, with considerably heightened crossing-over, especially to the left of the midpoint. It must not be forgotten that most of these experiments were not devised in such a way that the analysis is dependable. It seems quite probable that the experiments isolated into the three groups above are all modifications; but that they belong together in just three systems is not so certain, while it is certain that the remainder of the data, about three-fourths of the experiments, although accepted as "standard," is still heterogeneous.

STANDARD DATA.

We may now proceed to calculate the "standard" map, starting from the "standard" experiments above, each of which has been calculated for its recombination per cents.

PRIMARY AND SECONDARY DATA.

All the data bearing on the recombination for each two loci are collected, and are listed as "primary" or as "secondary" data. Secondary data are data upon the recombination for two loci when another locus situated between them is followed in the same experiment. Thus, in the four-point experiment A B C D, A–B, B–C, and C–D are primary recombinations, while A–C, A–D, and B–D are secondary. Sometimes data are used as primary when an intervening locus is followed, but this is usually in case the mutant of the intervening locus is not strictly classifiable. Thus, the recessive kidney overlaps the wild-type, and not all the flies that are genetically kidney can be separated out. Accordingly, the data from a spineless kidney sooty back-cross (table 36, p. 76) would be treated as follows: All the individuals would be used in calculating a ss–e^s value, and these data would be classed as primary, and would be used in the calculation of the primary bases of the map. The ss–k and k–e^s values would be calculated from the kidney flies only, and these data

would not be used in calculating the primary distances of the map, but only in placing the one locus k between ss and e^s at a point whose distances from ss and from e^s are proportional to the two values ss–k and k–e^s.

The division into primary and secondary data is to avoid the use of the same data twice in calculating the length of a section. Thus, if one had two experiments, each with 1,000 individuals, viz., the three-point experiment A B C, and the two-point experiment A C, from which to calculate a map, one should calculate the A–C distances from the A–C primary data weighted at 1,000, and from the A–B plus B–C primary data weighted also at 1,000. To use in addition the A–C data from the three-point experiment would be to use a given set of individuals twice, once as A–B plus A–C data, and again as A–C. That is, the data on the A–C distance from the three-point experiment would be weighted twice as heavily as the two-point A–C data. This distinction applies only in calculating map-distances, and secondary data are on an equal footing with primary in calculating recombination per cents, especially for the loci farthest apart.

SUMMARY OF STANDARD RECOMBINATION DATA.

The different sets of data listed together as primary data for the recombination per cent of a given two loci are then combined by addition, so that the total recombinations and the total number of individuals are known. From these totals a mean recombination per cent is found which has a weight equal to the total number of individuals of all the separate experiments. Similar calculations are made for the secondary data by itself and for all data, i. e., for the combined primary and secondary data. In table 2 are listed the mean recombination per cents for the various loci upon which there are standard data.

CONVERSION OF RECOMBINATION PER CENTS INTO CROSS-OVER VALUES.

Before using these standard recombination per cents in constructing a map, they must be converted into equivalent cross-over values by a correction equal to the number of cases of crossing-over per 100 that are not represented by recombination individuals. The amount of this correction is found by studying those cases in which intermediate points have been followed, that is, in all the standard experiments in which three or more points are followed.

LISTING AND SUMMARIZING OF DOUBLE RECOMBINATION DATA.

The first step in determining the amount of this correction is to calculate the amount observed in each experiment for the various loci. The method can be illustrated by an example. In table 149 are given the results of an experiment in which the five loci se, D, ss, e, and ro were followed. Only the multiple recombination classes and the total number of individuals (1881) need be considered. There are two parallel classes resulting from simultaneous crossing-over in the se–D and in the D–ss sections. These are the

se D ss e ro and the + individuals. Their sum (19) is the 1–2 double recombination class. The multiple recombination classes of table 149 are thus:

1-2	1-3	1-4	2-3	2-4	3-4	1-2-3	1-2-4	1-3-4
19	33	33	12	37	8	2	4	2

The total 1–2 double recombination is the sum of the 1–2 (19), the 1–2–3 (2), and the 1–2–4 classes, or 25 individuals in a total of 1881. But each of these 25 individuals represents two cases of crossing-over that would not have been represented by recombination for se and ss. There are, accordingly, 50 in 1881 or 2.7 per cent of total 1–2 double recombination. For the interval se–e the multiple cross-overs that are unrepresented by se–e recombination are: $2\times(19+33+12+2+4+2)$ or 144 in 1881, i. e., 7.7 per cent. It should be noticed that the triple cross-overs, 1–2–3, in which all three cross-overs are within the interval dealt with, are evaluated just as though they had been doubles. This is because, in a triple crossing-over, the first crossing-over gives recombination that would be detected, but the two succeeding crossings-over neutralize each other and do not give recombination. Similarly, the omitted se–ro crossing-over would be 300, or 16.0 per cent; the D–es 28 or 6.9 per cent, and ss–ro 20 or 1.1 per cent. The above five-point experiment has supplied us for six intervals with per cents which are the differences between recombination and crossing-over. All the standard experiments are thus reduced to data on the different component intervals, and the data bearing on each interval are collected from these experiments and listed together.

MEAN CORRECTIONS, AND CURVES FOR CONVERTING RECOMBINATION PER CENTS INTO CROSS-OVER VALUES.

The next task is to calculate a mean correction for each of the intervals for which data have been listed. The calculation of mean corrections offers exceptional difficulties that can only be met by a roundabout method of successive approximations. It is to be remembered that the main task is the recalculation of the skeleton of the map; that is, the making of new calculations for distances that are already known, but for which there are new additional recombination data, and also new data bearing on the relation between recombination and map-distance. Each such recalculation gives a closer approximation to what may be regarded as the standard situation. At the beginning of each recalculation one is in possession of a map giving old-standard distances, and a table of corrections giving the relation between old-recombination per cents and old-standard distance. The table of corrections can be put into the form of a series of curves (like those of fig. 1), of which the base-line is the map of the chromosome, and the curve originating at a given locus shows by its height above any other locus the amount by which the cross-over value is greater than the corresponding recombination per cent. Strictly speaking, a new line-map can not be calculated without a new set of correction curves, and conversely, a new set of correction curves can not be calculated without a

new line-map. But we may use the new recombination data and the old curves and obtain a half new map; and we may use the old map and the new recombination data and obtain a half new set of correction curves. Of the two—the old line-map and the old set of correction curves—the line-map is based upon more extensive data and upon data not subject to so great variation because of dealing with excessively small percentages. Since the old maps offer a more secure foundation for half new curves than do the old curves for a half new map, the half new curves are the first to be recalculated. From the half new map and the half new curves and the new recombination per cents and new double cross-over data three-quarters new maps and curves can be made, and so on until the approximation to a new map and a new set of curves is satisfactorily close.

Let us therefore return to the calculation of mean corrections for given intervals, a step in the calculation of half new curves, and show how this is done by means of an example—that of the Dichæte Hairless interval. We find listed an experiment of 645 individuals in which the intervening point is Delta and in which twice the number of observed doubles is 6 or 0.9 per cent of the total number of individuals. The old set of correction curves indicate that the loci Δ and H are so close together that there is no double crossing-over between them, but that between D and Δ there is double crossing-over equivalent to a difference of 2.4 between recombination per cent and cross-over value. The D Δ H experiment can not detect all the double crossing-over that occurs between D and H. The amount 0.9 actually observed is a minimum, and must be increased by a presumptive 2.4 to give a value (3.3) that would be found if all the doubles were detectable. Since only the 0.9 correction was supplied by the D Δ H experiment, while the rest was inferred from other experiments, if the above data are to be combined with data from other experiments in which all of the correction is detectable, they must not be given a weight equal to that of an experiment in which all the correction is self-supplied. Roughly, nine thirty-thirds of the correction was self-supplied, so that the weight given this experiment is roughly 25 per cent that of an ideal experiment. Since there were 645 flies in the experiment, the value 3.3 has a relative importance equivalent to an ideal experiment of some 160 individuals. Another experiment listed is D p H, in which there were 1,974 individuals with 1.6 as the self-supplied correction. But the old curves indicate that within the approximately D–p interval 0.5 per cent of undetected crossing-over was occurring, while in the p–H interval 1.2 was omitted. The total correction is thus 1.6+0.3+1.2 or 3.1, of which 1.6 or roughly 50 per cent was self-supplied. This experiment is, then, about twice as valuable as the previous one, and is weighted at some 50 per cent of its 1,974 individuals or at 890. Another experiment listed is D p ss H, with 2.5 of self-supplied and only 0.6 of inferred correction. The total value of 3.1 is thus to be weighted at about 80 per cent of its face value, or at 190 instead of 243 individuals. The other experiments on the D H interval are likewise corrected and weighted. Then each corrected correction is multiplied by its weight, and the sum of these products is divided by the sum of the weights to give a mean correction. Mean corrections are similarly calculated for each of the other intervals for which data are available, and the different mean corrections are listed together, each with its weight.

From the list of mean corrections and their weights, the set of half new curves of correction are to be constructed. The mean corrections are relisted according to the locus at the left end of the interval; for example, all the data in which roughoid is to be the point of origin of a curve are assembled. The various items within each set are then arranged in sequence according to the distance from the point of origin of the terminal locus for each interval, thus: ru–D, ru–p, ru–H, ru–e. The two terminal loci H and e are only 1.2 units apart, so that the ru–H and the ru–e data are expected to be very similar. The ru–H mean correction is 24.2 with a weight of 780. The ru–e mean correction is 29.7, with a weight of 1,800. By reference to the set of old curves it is seen that a line tangent to the curve at a point midway between these two adjacent loci has a rise of 90 units for each 100 units of progression. That is, the ru–e is convertible into ru–H data by subtracting 90 per cent of 1.2, viz., 1.1, from the observed ru–e data. The ru–e value of 29.7 becomes a ru–H value of 28.6, and is combined with the original ru–H value of 24.2 to give an aggregate mean correction of 27.3 with a weight of 2,580. There is a series of intervals originating at sepia and another series originating at hairy. Since these two points of origin are known to be very close together (0.5 apart), the two series can be combined into one series that has its origin at hairy, by converting each sepia interval into a corresponding hairy one, according to the slope of the curve at the terminal point. Similarly, the three series originating at ss, at bx, and at bx–b can be combined to give an aggregate series with origin at ss. And then the ss–H and the ss–e value within this aggregate series may be combined to give a single value for a ss–H interval. By this means scattered data are collected, so that their characteristics are more apparent.

Each condensed series of aggregate-mean-corrections is now plotted as a series of ordinates at the terminal loci, and the reliability of each such plotted point is indicated by its weight. Each of the curves can now be somewhat smoothed along its length by comparison of successive points with their weights, and especially by comparison of like regions for the different curves. Thus, the course of the curve originating at scarlet is well determined as far as ebony, beyond which no data are available. But the curve originating at Dichaete, just to the left of scarlet, and the curve originating at pink, just to the right of scarlet, are both well established for the region beyond ebony. The curve for scarlet is accordingly drawn to lie between these curves in a ratio determined by that observed where all three are reliable.

APPLICATION OF CORRECTIONS TO RECOMBINATION PER CENTS.

One is now in possession of half new curves of correction, similar to (but not identical with) those of figure 1. With these curves and the new tables of recombination per cents (table 2) a line-map can be constructed that is somewhat better than half new. Each of the primary mean recombination per cents of table 2 is converted into the corresponding cross-over value by its individual correction, which can be read off directly from the half new curves.

CALCULATION OF THE LOCATIONS OF THE GENES.

The locus in the third chromosome that appears in the greatest volume and variety of data is Dichaete, so that Dichaete is the primary base of reference.

or anchorage, of the map. The second locus in point of importance is Hairless, together with its alternate ebony, for the data concerning these two loci are convertible, the one into the other, by knowledge of the distance between and of the slopes of the correction curves. The third locus in point of importance as a base of reference is claret, with its alternate Minute. The first positions to be calculated are, then, those immediately referable to D, to H, and to ca. The procedure can be illustrated by following the calculation of the loci about Hairless. The first locus to be calculated is that of ebony, which is to the right by a distance that is directly observed as 1.2 units, in experiments aggregating 3,992 individuals.

The next locus to be calculated is that of band. The data are a direct H–bn value of 2.4 with a weight of 2,370, and a Δ–bn value of 6.8 based on 28 individuals. But to locate band with reference to Delta, the locus of Delta must be known, and conversely, to locate Delta by use of all the data the locus of band must be known. This difficulty is met by using half the weight of the Δ–bn data in locating bn and half in locating Δ. The old map gives a position for Δ at 3.3 units to the left of H, which would indicate a locus for bn 3.5 to the right of H. Weighted at 140 and combined with the direct H–bn data gives a provisional location at 2.5 to the right of H. The location of Δ is calculated from a direct observation of 2.9 to the left of H with a weight of 4,469, a Δ–e value of 4.4 convertible to a Δ–H value of 3.2 with a weight of 861, a Δ–bn value of 6.8 convertible to a Δ–H value of 4.4 (assuming the provisional locus of 2.5 for bn) weighted at half of 281 or 140, and finally from a similar use of half the weight of a Δ–wo value of 10.3 based on 310 and a provisional locus of wo at 6.8 to the right of H. The combination of the above data gives a provisional location for Delta at 3.3 units to the left of Hairless.

Similarly, a provisional or half new location is calculated for white-ocelli. The locations of three of the five loci considered are mutually interdependent to the extent to which data having inferred locations are used in each. These loci can now be recalculated, using the half new locations instead of the old, where inferences are needed. The new data are not greatly different from the old, so that this second approximation gives locations that are acceptable. This whole group of five interrelated loci is then used as a base in calculating the position of all the loci from the gap to the left of spineless to the gap to the right of white-ocelli. A similar block of interrelated loci is centered about Dichæte, and a third about claret. The rather isolated locus roughoid, to the extreme left, is tied on to the Dichæte group by reference to sepia, to hairy and to Dichæte, whose positions within the Dichæte group are known. The claret group is tied to the Hairless group by all the values that span the gap between white-ocelli and rough. Of these the most important is ebony-rough, though white-ocelli-rough has also a very large weight. Lastly, the D and the H groups are tied together by all the primary data in which one locus is located in the group about Dichæte and the other locus is in the group about Hairless. Of these, the most important are p–ss, D–ss, si–ss, p–H, p–e, D–H, and D–e, though there are very many others of lesser weights. This completes the calculation of the skeleton of the half new map.

Starting with the half new map and the new table of corrections, the whole procedure of calculating mean corrections for each interval and subsequently combining these into curves is gone through with again. With these three-

quarters new curves and the new table of recombination data, a three-quarters new skeleton map is constructed. Similarly, one more approximation is made throughout, which gives the curves and line-map of figure 1.

However, the curves of figure 1 have been checked, and altered somewhat, by comparison with the directly observed percentages of recombination listed as the total for primary and secondary data in table 2. This checking was especially useful for the values in the upper and in the right halves of the field of curves, that is, where long distances are involved.

The greatest usefulness of the curves of correction of figure 1, aside from their necessity in constructing the line-map, is that by means of them any observed recombination per cent can be easily translated into terms of map-distance, or conversely, any given map-distance can be converted into an expectation for recombination.

It should be noted especially that the curves originating at the various loci are specifically and rather widely different from one another. This becomes strikingly apparent from a comparison of ordinates at equal distances from the origins of, for example, the p and the ss curves, or the ru and the D curves. But these differences are themselves in an orderly system, which is connected with the regional differentiation of the chromosome. The length of section, measured in units of map-distance, within which a double cross-over may occur, is shortest in the region about pink and longest in the region near white-ocelli, and has a characteristic value for each region of the chromosome, except that the values are probably symmetrical about a point lying near warped. Accordingly, all the curves having their origins to the left of pink make decided rises as they pass this mid-region, those originating near-by at the left have rises early in their course, while those originating further to the left rise progressively later in their courses. To attempt to express by a single curve or formula the relation between map-distance and recombination would only obscure the real situation, and the result would be as meaningless as a map based upon all crossing-over data, irrespective of known modifiers of crossing-over. At present the most satisfactory representation of the relationship between recombination and map-distance is one that gives for each particular chromosome and for each section of that chromosome the observed relationship. From such careful, detailed, region-by-region studies a more general statement and formulation should in time emerge.

The recombination per cents corresponding to particular map-distance can be put into the form of curves (fig. 2), so that the relation between any given recombination per cent and cross-over value can be read off directly. The base-line of figure 2 is the new line-map, with the first-class mutants represented with greater conspicuousness than the second-class.

The 45° lines from the different loci enable one to read off directly the cross-over value for any two loci, instead of obtaining it by subtraction. The difference between the slanting line and the curved line originating at the same locus can be read off directly as the correction identical with that of figure 1. These various features make the type of map shown in figure 2 the most generally useful of any so far devised.

A third form of map that is very useful in planning experiments is the evaluation map given in figure 3. The loci are divided into four classes, first, second, and third, and, in the fourth column, those no longer extant. The

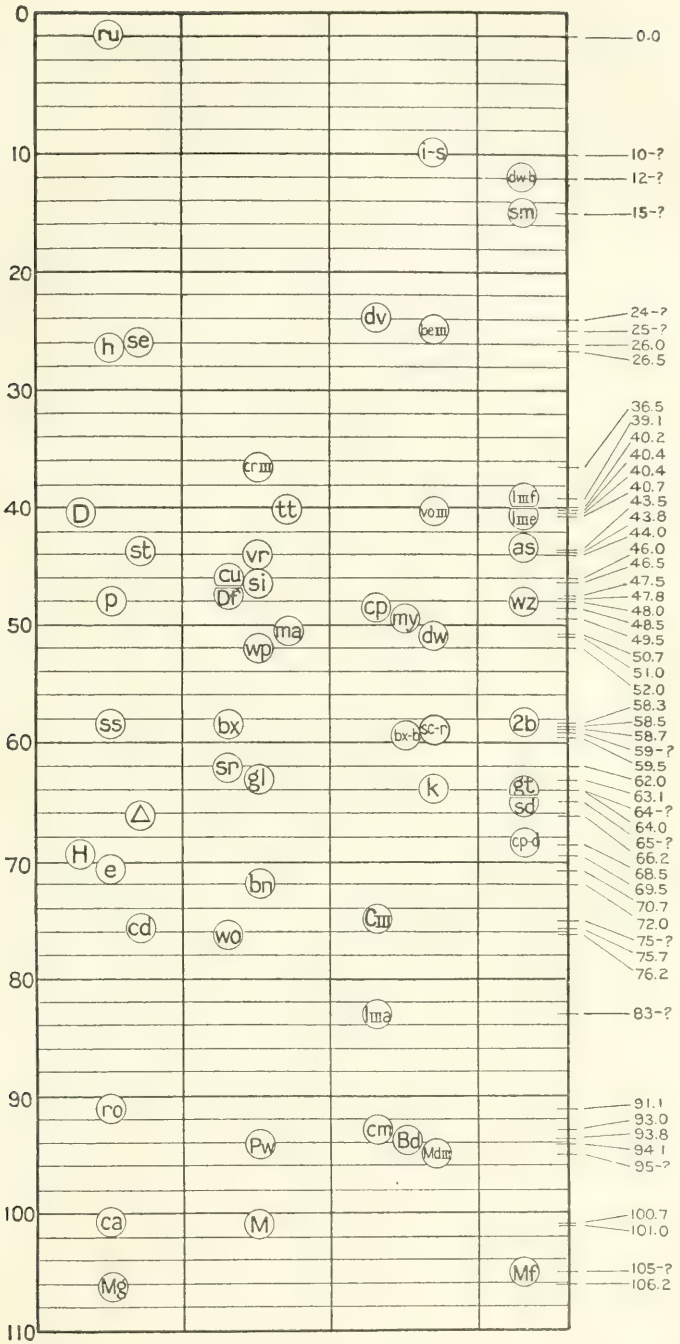


FIG. 3.—Line-map and evaluation map of chromosome-III. The loci nearest the left margin are those most useful. Those farther to the right are progressively less useful. Those in the fourth class are no longer extant.

order from right to left is that of increasing importance for experimental work. The locations for the first and most of the second class loci have been calculated as part of the skeleton map of figure 2. The locations for the third and fourth class loci have been made later by interpolation according to the method described in connection with kidney on page 20.

GENERAL DISCUSSION OF MAPS.

There has been some misconception as to the purpose for which the maps are made. They are based upon all data in which no obvious or large crossing-over variations are present, and are a sort of composite picture of the most frequent types of result. They furnish a basis for prediction as to the most probable values that an untried experiment will give. Special cases have been met with in our work where different crossing-over frequencies were present, due either to environmental differences in age, or in temperature, or to the presence of genes that modify crossing-over in a particular way. Sturtevant (1919) has described an extreme case of this sort in which nearly all crossing-over in the second-chromosome was suppressed. Similar examples for the third-chromosome have been met with, and are described in the present communication. Several in the X–chromosome are also known to us, and Detlefsen has recently found another case of the same kind. One of our own cases, like that of Detlefsen, appears to have a polygenic basis, and in both cases selection shifted the recombination per cents in the direction of selection. The results are in accord with the effect of selection in polygenic cases in general. There is nothing in any of these results to indicate that selection has acted in any other way than by isolation and combination of modifying genes. In none of these cases is the order of the mapped loci changed. Even in cases where practically all crossing-over is suppressed, the chromosomes involved, when set free from their modifying genes, give the standard amount of crossing-over. In his two papers, especially in the first one, Detlefsen seemed to imply that changes in crossing-over frequencies, such as those he found, affected the character of our conclusions, but in fact, there was nothing new in principle in his results which we had not already obtained, nor did they affect the value of the maps for the purpose for which they were made.

At present the III–chromosome map is 106 units long. In the most recent maps the X–chromosome has a length of 70 units, the II of 107, the III of 106 units, and the IV of 1 unit. These lengths are in approximately the same proportion as the lengths of the oögonial chromosomes in metaphase plates. If the length of the X chromosome is taken as unity, the lengths are proportionally 1.0 : 1.7 : 1.5 : 0.1. Similarly, if the map of the X chromosome is taken as unity, the lengths of the maps of the chromosomes are in the proportion 1.0 : 1.5 : 1.5 : 0.1. This rather close coincidence in proportion means that a unit of map-distance corresponds on the average to approximately the same length of chromosomes in each of the four chromosomes. But, as will presently be shown, a unit of map-distance may correspond to quite different lengths of chromosome within the same chromosome.

The number of recorded mutant loci in the different linkage groups are also roughly proportional to the lengths of the chromosomes. Because sex-linked mutations show themselves immediately in males they are more likely to be

discovered than are non-sex-linked mutations, even if they occur with the same frequency. The relatively greater number of recorded sex-linked mutants is probably to be explained in this way. For autosomal mutants the likelihood of discovery is the same for all the chromosomes, and the number of II, III, and of IV–chromosome mutants are observed to be roughly in proportion to the length of the actual chromosomes. This means that, on an average, sections of chromosome of equal length are equally likely to give mutations.

An examination of the map of chromosome III shows that the mutant loci are not evenly distributed throughout the length of the chromosome, but that in the neighborhood of pink there is a cluster of mutated loci. It may appear, then, either that mutations in the III–chromosome are not of equal frequency for various sections of equal lengths, or that the region near pink represents a relatively long section in which there is relatively little crossing-over. Other independent evidence, as will be shown later, makes the latter alternative more probable.

Conversely, a map-region in which there are few mutant loci probably represents a chromosome region in which crossing-over is relatively frequent, so that the map-distances between the loci are relatively greater than the corresponding distances in the chromosome; i. e., a unit of map-distance for such a region corresponds to a relatively short length of chromosome. In the map of chromosome III there are two such regions with relatively few mutant loci, namely, one to the left of sepia and the other to the right of white-ocelli. These, as stated above, probably correspond to regions of the chromosome in which crossing-over is relatively frequent.

The III–chromosome is bent at its middle as it lies in the metaphase plate; and, as it moves towards the pole of the mitotic figure, the spindle fiber is also seen to be attached to this mid-point. If the map of the third chromosome represents approximately all of the length of the III–chromosome, its mid-point, at 53, should correspond roughly to the mid-point of the actual chromosome, i. e., the mid-point of the third chromosome corresponds to some point between pink and spineless.

Also, if mutation is as likely to occur in one half of the III–chromosome as in the other half, then the median locus of the map should correspond roughly to the mid-point of the actual chromosome. The median locus is that of warped, which is mapped at 52, between pink and spineless.

In the II–chromosome, the mid-point, as determined by the middle of the map, and by the median of the mapped loci, is at purple. Purple has been found to be a critical point in the action of cross-over modifiers. For example, the factor CIIL suppresses nearly all crossing-over to the left of purple and leaves crossing-over to the right of purple unaffected, while, conversely the modifier, CIIR eliminates most of the crossing-over to the right of purple, but is without effect on crossing-over to the left of purple. It appears, then, that cross-over modifiers probably furnish an independent means of determining the mid-point of the chromosome. In the III–chromosome the cross-over modifier known as CIII eliminates nearly all crossing-over throughout a section extending from a point a few units to the right of pink to the right end. This determination of the mid-point agrees with that indicated by the other two methods.

It has been observed that the region about purple and also that about pink are most susceptible to changes in crossing-over due to abnormally high or low temperature.

These same two regions are the ones most affected by age-difference. Crossing-over is high in the first output of eggs and gradually decreases as the fly gets older and rises again in still older flies.

The regions about purple and about pink differ from the other regions in still another respect, namely, in that the average length of the sections included between double cross-overs in these regions is shortest. Stated in another way, coincidence is highest in these regions.

The question may be asked: Is there anything known in regard to the middle region of these two chromosomes that will account for the above peculiarities? If these chromosomes are loops at the time of synapsis, as in the well-known bouquet stage, and if synapsis begins at the ends of the loops and proceeds towards the mid-point, the distribution of crossing-over might be expected to be symmetrical with respect to the ends. The same result would follow if synapsis and crossing-over were initiated at the tip of the loop and traveled toward the ends. Since the two limbs are equally long, the mid-point of the chromosome would be the center of symmetry of the chromosome. Points equally distant from the mid-point (or from the ends) should have the same characteristic behavior with respect to crossing-over. Crossing-over might well be different at different levels; the levels at which crossing-over is relatively most frequent would appear on the map as regions where mutant loci are relatively infrequent, as pointed out above.

The X–chromosome differs from the II– and III–chromosomes in being a straight rod. The spindle fiber is attached at one end and not at the middle as in the II– and III–chromosomes. It might be compared with one of the limbs of a bent chromosome. Actually, there is a massing of mutant loci at the left end, and there is a space relatively free of mutant loci about two-fifths the distance from the right end. The left end is also more susceptible to cross-over variations. These relations may be comparable with those in the other two chromosomes, if, in the female, the two X's are not disposed in the form of loops, and if synapsis and crossing-over begin at one end only.

II. GENERAL ACCOUNT OF MUTANTS.

WITH; SUPERWITH.

(Figure 4.)

ORIGIN OF WITH.

The first work done with *Drosophila* in the Columbia Laboratories was the attempt made by Morgan during the fall and winter of 1909-10 to produce mutations through treatment with different chemicals, varying food conditions, and finally by physical agents such as radium. These attempts produced no definite result (see section on Beaded, p. 37); but as a side issue, Morgan undertook a selection experiment to obtain a stock all the members of which should have a dark trident-pattern on the thorax.

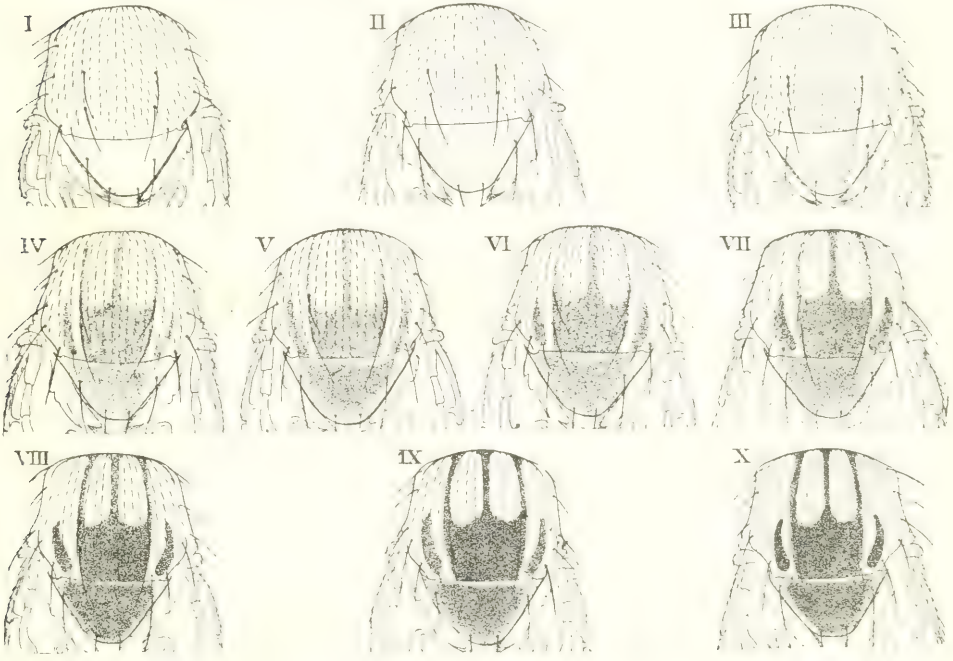


FIG. 4.—The grades of pigmentation of the trident pattern of "with" flies.

When the series of treatments was begun in the early fall of 1909 it was observed that the trident-pattern on the thorax of some of the wild stocks that were being used varied both in the intensity of the pigment and in the extent to which the pattern was spread over the thorax and scutellum. The degree of pigmentation and the extent of the pattern seemed to vary together.

It is probable that the only real variation was in the amount of pigmentation, and that the pattern was the same throughout, but was not discernible,

except to the extent that pigment was developed. In a small proportion of the flies the pigmentation was so faint that all traces of the trident-pattern disappeared. These flies were called "without" (without trident-pattern), and are represented by grade I of figure 4. Roughly, a third of the flies had a barely discernible flush of pigment on the scutellum (grade II, fig. 4). Slightly fewer than a third had an easily seen scutellar sootiness, and the pentagonal area was quite sharply defined (grade III). Flies of these last two grades (II and III) constituted the bulk of the flies of all wild stocks examined, but in addition there were present smaller proportions of flies with still darker pattern, namely, grades IV, V, and VI. Grade IV is characterized by the presence of three faint prongs at the anterior angles of the pentagon and a pair of side prongs flanking this trident-pattern. Grades V and VI represented stronger and darker lines throughout. The darkest flies observed in stocks of wild flies were like those represented by grade VI, which, however, occurred as only a small percentage of the flies.

THE TRIDENT-PATTERN OF WILD STOCKS.

At various times careful examination has been made of several different wild stocks and the number of flies of each grade determined. These stocks proved to be all quite similar, and the distribution observed (October 1911) in two

TABLE 3.—*The grades of "with" in wild stock.*

Oct. 12, 1911.	I.	II.	III.	IV.	V.	VI.	Total.
Fal. 1910.....	131	192	140	66	27	.....	556
Fal. 1911.....	49	393	302	209	79	26	1058
Total.....	180	585	442	275	106	26	1614
Per cents.....	11.1	36.3	27.4	17.0	6.6	1.6	100.0

"Falmouth" stocks may be taken as representative (table 3, and graph a in fig. 5).

"WITH" STOCK.

Morgan selected throughout the fall and winter of 1909-10 for increased trident-pattern, with no certain progress until January 1910, when a few flies were found that were considerably darker than any hitherto observed.

By breeding from these darker flies a race was quickly obtained which showed no individuals as low in grade as those in classes I and II and very few in class III.* This stock was called "with," and mass selection in it was continued for about a year ($30 \pm$ generations), with no apparent result further than possibly to decrease the numbers in the lower grades. It appears that the various grades observed in the stock prior to January 1910 were merely the normal fluctuations of the wild-type pattern, and that a definite mutation had then occurred which gave rise to higher grades and a characteristic new

* The scale of grades was not devised until the summer of 1911, and the account of the previous work is referred to that scale only as a matter of convenience in description.

distribution. A census taken of this new "with" mutant population (October 1911) revealed the condition shown in table 4 and by graph b of figure 5. The

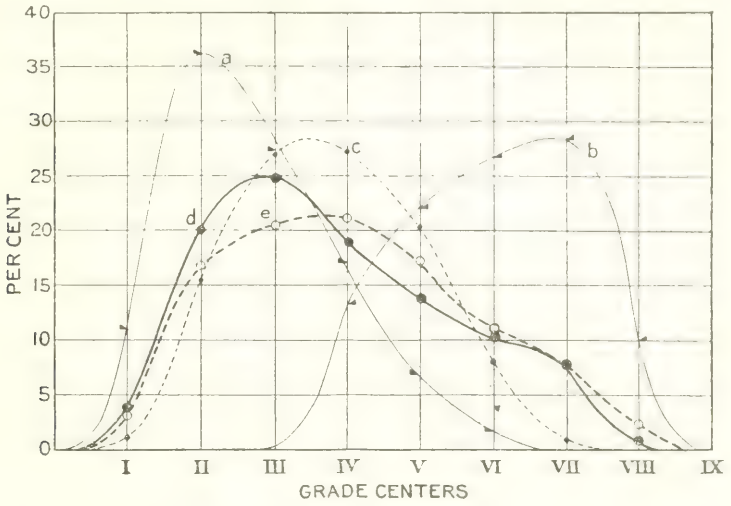


FIG. 5.—Frequency distribution of the grades of "with" occurring in parental wild stock (a), parental "with" stock (b), the F_1 hybrids (c), and the F_2 population (d). Curve e gives an F_2 expectation calculated by combining the parental curve a with the parental curve b and with twice the hybrid curve c.

most numerous class in the "with" stock was VII, which was not represented at all among the wild flies.

TABLE 4.—*The grades of "with" in cultures of various "with" stocks.*

Oct. 12, 1911.	IV.	V.	VI.	VII.	VIII.	Total.
Stock w	142	242	297	304	127	1112
Stock x	78	110	130	145	39	502
Stock x_1	91	151	164	174	54	634
Stock y	22	62	93	89	50	316
Stock z	32	30	45	61	6	174
Total	365	398	729	773	273	2738
Per cents	13.3	21.8	26.6	28.3	10.0	100.0

THE F_1 RESULT OF THE CROSS BETWEEN "WITH" AND THE WILD STOCK.

The classifications of tables 3 and 4 were made preparatory to a cross between these two lines. The F_1 flies from the cross of "without" wild flies to "with" flies of the mutant stock were in the main intermediate in grade between the two parental types (table 5, and graph c of fig. 5). The mode of the F_1 was at grade IV (mathematical mode 3.7). There were almost no flies that did not show a faint pattern (grade I = 1.2 per cent). At the other extreme there were no flies that were as dark as grade VIII and only a very few (1.1 per cent)

as dark as grade VII. A comparison of the F_1 distribution with that of each parent (see fig. 5) shows that, as judged by the means, modes and in the overlap, the F_1 population resembles the wild parent more closely than the "with" parent.

TABLE 5.—*The grades of "with" in F_1 from Falmouth × "with."*

Nov. 6, 1911.	I.	II.	III.	IV.	V.	VI.	VII.	Total.
A. "with" ♂ × + ♀	.....	11	37	45	50	30	6	179
C. "with" ♂ × + ♀	32	119	258	265	186	94	20	970
B. "with" ♀ × + ♂	.....	55	76	75	62	21	.....	289
D. "with" ♀ × + ♂	.....	208	326	315	221	73	2	1145
Totals.....	32	393	697	700	519	218	28	2587
Per cents.....	1.2	15.2	26.9	27.1	20.1	8.4	1.1	100.0

Upon the basis of these three curves, "with" would be ranked as a recessive. Since "with" is the mutant type, and since the F_1 shows considerable similarity to "with," "with" should be judged a dominant, or a "partial" dominant. Besides these two criteria there is another by which the dominant-recessive relation may be defined (and it is this one that we have adopted for *Drosophila*), namely, that of practical convenience. We call the mutant type a dominant or a recessive according to whether in experiments a greater degree of accuracy or of ease in classification is obtained by its use as one or the other.

THE F_2 FROM THE CROSS OF "WITH" TO WILD.

The F_2 results (table 6, curve d, fig. 5) gave a range of grades that included the faintest "withouts" of the wild grandparent and the darkest "with" of the other grandparent.

TABLE 6.—*The observed distribution of grades of "with" in the F_2 from Falmouth × "with."*

Nov. 20, 1911.	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	Total.
A.....	1	61	75	89	73	58	50	12	419
B.....	.....	60	62	89	94	73	55	17	450
C.....	58	217	330	167	125	76	67	2	1042
D.....	59	283	306	233	140	106	61	1	1189
Total.....	118	621	773	578	432	313	233	32	3100
Per cents..	3.8	20.0	24.9	18.7	14.0	10.1	7.5	1.0	100.0

In shape the F_2 curve presented its main mode at grade III and an indication of another mode at VII.

If our assumption is correct that the "with" stock differed from the wild because of a single mutant difference, the F_2 population should be composite, one quarter being like the wild parent, one quarter like the "with" parent, and two quarters like the F_1 type. On this basis an expectation for the F_2 that should result from the given P_1 stocks and the known F_1 distribution can be calcu-

lated by taking for each grade the sum of its percentage frequency, as wild-type parent, as "with" parent and twice its frequency of occurrence among the F_1 offspring (table 7). This result should be reduced to the basis of 100 by divid-

TABLE 7.—*The distribution of "with" in F_2 expected from the observed P_1 and F_1 distributions.*

	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	Total.
Wild.....	11.1	36.3	27.4	17.0	6.6	1.6	.....	.....	100.0
"with".....	.....	.....	.....	13.3	21.8	26.6	28.3	10.0	100.0
$2 \times F_1$.....	2.4	30.4	53.8	54.2	40.2	16.8	2.2	.....	200.0
Total....	13.5	66.7	81.2	84.5	68.6	45.0	30.5	10.0	400.0
Per cents.	3.4	16.7	20.3	21.1	17.2	11.2	7.6	2.5	100.0

ing through by 4 in order that the distribution be directly comparable with the observed F_2 result.

The graphs of figure 5 show that the observed F_2 result (curve *d*) is in good accord with the expectation (curve *e*). The observed F_2 differed from that calculated in that there was slightly too great a proportion in grade VII (which corresponds to the mode of the "with" stock) and also a similar excess in grade II and III (which corresponds to the bulk of the wild stock). These differences suggest that in the F_2 the "with" flies had proved more sharply defined as the recessive type and that the heterozygotes as a class resembled the wild parent somewhat more strongly than they had in F_1.

It should be noted that had "contamination of genes" been acting, the F_2 should have deviated from the calculated in the direction of the F_1 result, so that the curve of the observed F_2 should have lain between the calculated F_2 curve and the F_1 curve. Instead of this the actual deviations were in the opposite direction.

SUPER-WITH.

The "with" stock used in the preceding experiments had undergone somewhat over a year of mass selection with little or no observable progress. However, after some few months of selections, decided progress had been obtained in a sister line. A certain culture (E, November 1910) was seen to con-

TABLE 8.—*The distribution of "with" patterns in line: E11b 2 2 1 4 1 8 6 1 1 7 7 9 b b A A 5 A A.*

Sept. 14, 1911.	VII.	VIII.	IX.	X.
Totals.....	53	65	70	104

tain individuals of still darker grades (IX and X) and selection soon established a "super-with" population. This E line then proved as immovable as before, although selection was practiced continually. At the end of 23 generations a census of this line (September 1911) showed the condition of table 8.

This stock was lost before tests were carried out, but we can judge from its sudden manner of origin and subsequent stability that a second mutation had occurred. Whether this second mutation (super-with) was a modifier of "with" or an allelomorph of "with" was not determined.

CHROMOSOME CARRYING "WITH."

It was evident that "with" would not be useful in linkage experiments on account of the inability to make a complete separation between the "with" and the heterozygous "with" types. Accordingly, no further work was done with the mutation, except to determine in which chromosome its gene is situated. This was done (December 1913) by means of the F_2 of the cross of pink by "with", which produced no pinks that were surely "with" and no sure "withs" that were pink. This result was a consequence of the fact of no crossing-over in the male and proved that the locus of "with" is in the third chromosome.

LOCUS OF "WITH."

The recessive mutant "band" arose in "with" stock, and the F_2 of the crosses of the double form ("with" band) to maroon and to pink furnished other evidence that "with" is in the third chromosome (see p. 79). It was also observed that the double recessives, maroon band and pink band, obtained by crossing-over, were of a different type of band than the original, and this difference was of such a nature that it could be readily explained by assuming that the "with" character had been lost by the crossing-over. This means that the "with" locus is to the left of that of band, and either to the left of pink or not far to the right of pink.

EVALUATION OF "WITH."

Because of the increase in the number of our stocks of mutations to unwieldy proportions, the stock of "with" was discarded in June 1914, along with several others which were judged to be of little further value. The stock of "super-with" was lost through sterility or inviability.

OLIVE–III.

Selections for dark trident-pattern, parallel to those which gave rise to the stocks of "with," "without," and "speck," resulted in a stock called "olive" (April 1910). The "olive" stock was found to be pure for a character called "speck," * which is a minute dense pigment-spot in the axil of the wing.

During the fall and winter of 1912 a good deal of breeding work was carried out to determine the relationship between "speck" and "olive," and to find to which chromosome group they belonged. "Speck olive" flies were outcrossed to the second-chromosome recessive "black," and 10 F_2 cultures were raised (tables 4 and 5, p. 131, Carnegie Publication No. 278). The F_2 results (Wild-type 619, speck 19, "olive" 479, speck "olive" 447, black 590, black

* An account of these selection experiments has already been given in Carnegie Publication No. 278, on pages 128 ff (speck), and 135 ff (olive-II); also the preceding section on "with" and "super-with" gives further details.

speck 0) showed that speck was a second-chromosome recessive, since the double recessive black speck did not occur. Nearly all of the speck flies were "olive," which showed that there was present a second-chromosome recessive (olive–11) closely linked to speck. But also there were present as many flies (479) that were simply "olive." The total number of "olives" was thus far too large to be explained by a single recessive gene for "olive." The ratios were in accord with the assumption of two independent genes for olive, one in the second chromosome and another in some other autosome.

Speck olive flies were also outcrossed to wild, and 15 F_2 cultures were raised (tables 2 and 3, p. 130, Carnegie Publication No. 278). The F_2 results (Wild-type 1,556, speck 66, "olive" 529, speck "olive" 706) showed that the ratio of not-olive to olive 1,622 : 1,235, was almost exactly the 9 : 7 ratio expected on the basis of the two independent recessives giving similar effects and not markedly cumulative in effect.

The relation between speck and "olive" showed that one of these genes was in the second chromosome. The other was known not to be sex-linked and was assumed to be in the third chromosome. No tests of this were made, and the stock was later discarded. The probability that the other olive gene was in the third chromosome rather than the fourth is about 15 : 1.

BEADED (Bd).

(Figure 6, page 38; Figure 20*b*, page 152.)

ORIGIN OF BEADED MUTANT.

During the fall and winter of 1909-10 Morgan subjected cultures of *Drosophila* to treatments by various acids, bases, salts, sugars, and different foods and culture media in an effort to induce mutation. In these experiments no mutations were found that had been induced by the chemical treatments or that had arisen spontaneously. In May 1910 the effect of the rays from radium was tried on a culture containing flies in all stages of development (Morgan, 1911). Among the offspring, one male occurred whose wings showed a few shallow marginal scallops which removed the marginal bristles, the marginal vein and slight amounts of the blade of the wing. The appearance of the remainder of the margin led to the name "Beaded" for the character. Subsequent work with radium gave no indication that the appearance of Beaded was due to the use of radium.

STOCK OF BEADED.

This "Beaded" male was bred to several of his sisters and in the next generation there were a few Beaded flies, mainly females. The Beaded flies were bred together and produced a slightly higher proportion of Beaded offspring. Mass selections and non-virginity probably kept the proportions of Beaded lower at first than it would otherwise have been.

SELECTION OF BEADED.

From the first the character Beaded has been one of the most variable worked with. Often one wing would be entirely normal and the other present but a single barely discernible scallop. In general, there was a medium high correlation between the two wings, for rarely did a normal wing occur with

a highly Beaded mate. In the first generations the extent of beading seldom surpassed that shown by figure 6b. Continued selection increased both the percentage of Beaded flies and the extent to which the character was

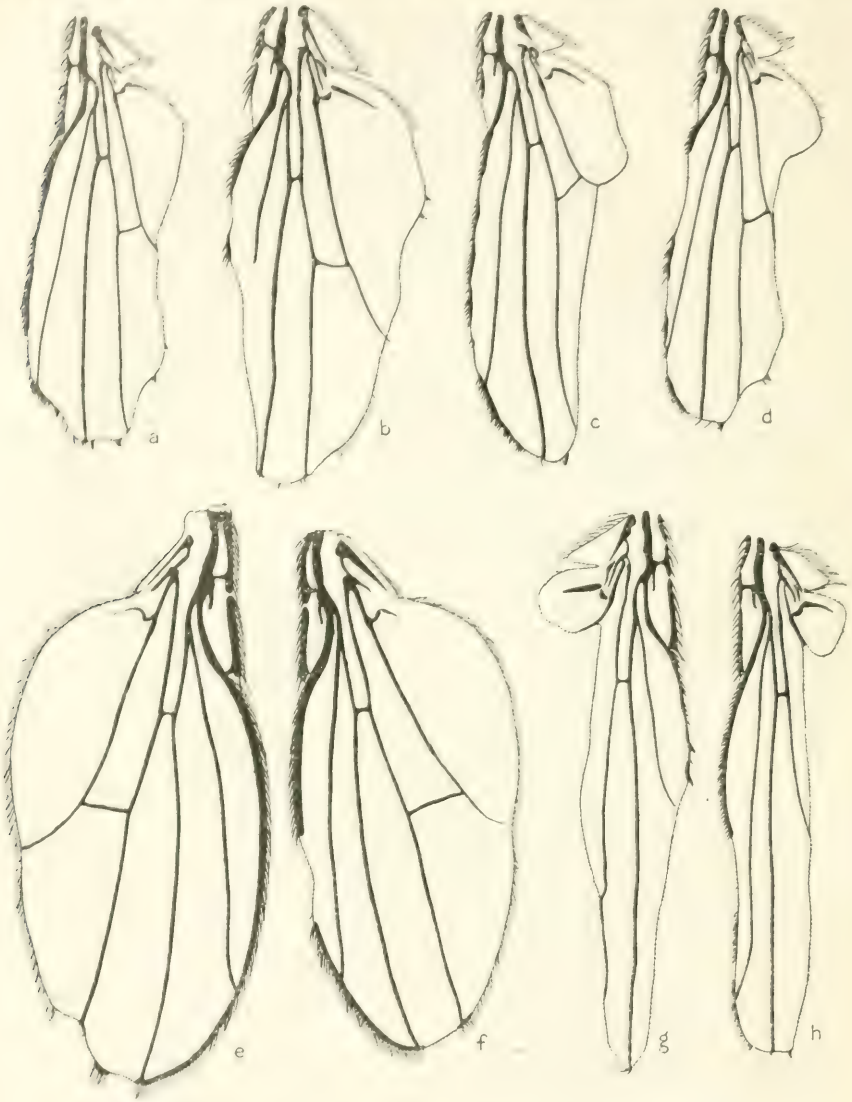


FIG. 6.—Beaded wings. (a, b, c, d) Average Beaded of balanced stock. (e, f) Slight Beadeds of F_1 from outcrosses. (g, h) Extreme Beadeds of selected stock.

developed in the extreme individuals. Progress was apparently nearly continuous, the large amount of fluctuation in the character effectively concealing all except large steps. The selection, too, was by means of mass cultures and was not recorded each generation in terms of grades, etc. There were only two

occasions when progress was quite plainly discontinuous. The first of these was fairly early in the selection and resulted in a new line in which the Beadeds were quite extreme in type and constituted about two-thirds of the population. The second decided advance occurred (about December 1911) after nearly two years of selection (some 50 generations) and resulted in a stock nearly every individual of which was Beaded instead of only about two-thirds, and most of these Beaded flies were of the extreme type.

THE LINKAGE METHOD OF THE ANALYSIS OF MULTIPLE-GENE CASES.

The discovery of specific modifiers of mutant characters in *Drosophila* and an increasing knowledge of the prevalence of modifiers and multiple-gene cases in several forms led to the suggestion that the history of the Beaded stock might be explained on that basis. By means of its linkage relations, cream–II had been proven to be due to the action of a specific gene which differed not at all in inheritance from other genes, but whose only observable effect was the dilution of eosin eye-color. Muller had developed in theory this method for the analysis by linkage of multiple-gene cases, and had showed by this method that it should be possible to give a demonstration of the number and qualities of all the genes involved in cases so complex that previous methods could at best only indicate the probable condition.

DEXTER'S ANALYSIS OF BEADED.

Dexter studied the case of Beaded, using the method of linkage extensively. It was known that Beaded is a dominant, i. e., that the cross of Beaded to unrelated flies such as those of a wild stock produced Beaded flies among the progeny. The type of these F_1 Beadeds was not as extreme as that of the selected stocks used as parent; and the percentage was low, seldom reaching 50 per cent. Of about 60 such matings carried out by Dexter only 2 gave as high as 50 per cent of Beaded in F_1, while the most frequent percentages were about 15 and about 30.

AGE-FACTOR IN BEADED.

Dexter found that the last counts of a culture gave fewer Beaded flies than the first counts, and that second broods probably gave higher percentages than first broods. This would indicate a rhythmical change with age, somewhat similar to that known to hold for linkage (Bridges, 1915).

ENVIRONMENTAL FACTORS IN BEADED.

That the great variation in percentages was also largely dependent on environmental factors was suggested by tests that showed that in very wet bottles there was a greater proportion of Beaded than in very dry bottles, and that cultures in which the food was decidedly acid produced fewer Beaded than others made alkaline.

THE BEADED GENE AND THE LETHAL EFFECT.

That the Beaded and non-Beaded F_1 flies were of different genetic constitution seemed clear from the different F_2 results produced by different types

of F_1 mating. Beaded × Beaded gave from 50 to 70 per cent of Beaded offspring. Beaded × wild-type gave mainly 25 to 50 per cent Beaded offspring, and wild-type × wild-type gave usually no Beadeds, but occasionally 25 to 50 per cent of Beaded offspring. It seems certain that in these latter cases one of the wild-type parents had been of the same gentic type as the Beaded flies and represented a fluctuation of the Beaded character.

Another striking fact was that these F_1 Beadeds, that could only be heterozygous for the Beaded gene, gave almost as high percentages of Beaded as did the Beadeds from the pure breeding-stock, when outcrossed to wild flies in each case. This test was repeated for several generations with the same result, namely, that the percentage of Beaded was about 25 in some lines, but in other lines only about 5 per cent.

This behavior of Beaded led to the belief that the Beaded stock also was heterozygous, and that a *lethal* gene was operating to keep it heterozygous. Dexter compared Beaded to the yellow mouse, where every yellow mouse is a heterozygous dominant. Dexter was then faced with the difficulty of explaining a permanently heterozygous stock that bred true, for while a stock of yellow mice never breeds true, a stock of Beaded had been obtained that did. Dexter attempted an explanation by assuming that the heterozygosity was in a lethal gene distinct from the dominant Beaded gene, and that the pure-breeding stock was homozygous for Beaded but heterozygous for lethal. Although Dexter's explanations came near to the actual case, they failed of being complete, as we shall see later.

DEXTER'S ANALYSIS BY MEANS OF LINKAGE—THE CHROMOSOME INVOLVED.

The first step in the analysis of the constitution of the Beaded stock by means of the linkage method was to determine which chromosomes carried mutant genes contributing to the extent of the development of the character and the proportion of flies showing the character. Dexter raised F_2 cultures from crosses of Beaded to various characters whose genes were in the first-, second- and third-chromosomes respectively.

LOCATION OF THE BEADED GENE.

It became apparent that the manifestation of Beaded was principally dependent upon a gene or genes located in the third chromosome, since very pronounced linkage was observed in the different crosses between Beaded and the third-chromosome recessives sepia, pink, maroon, and ebony. The third-chromosome effect was found to be very largely due to a gene located quite far from pink (40 per cent of recombination in such tests, table 2), and to be in the region including ebony. This principal gene will be called simply the Beaded gene.

BEADED-INTENSIFIER-III.

From the pink Beaded recombinations that occurred in the F_2 of the cross of pink ebony × Beaded a stock of pink Beaded was derived. When individuals from this stock were out-crossed to white, to black, and to wild flies, there were produced in F_1 markedly fewer Beaded offspring than were given in

like out-crosses of the parent Beaded stock. This fact is interpreted as due to the absence from the pink Beaded stock of a dominant intensifier present in the selected Beaded stock. That the fewness of the pinks in these F_1 crosses was not due to an inhibiting effect of pink is plain from the high percentages of F_1 Beadeds that had occurred in the crosses of Beaded pink ebony. The locus of this intensifier of Beaded is to the left of Beaded, since it was removed by crossing-over between pink and Beaded.

BEADED-INTENSIFIER-II.

In the F_2 of the crosses to the second-chromosome recessives black, purple, curved, and arc, it became apparent that the production of the Beaded character was not independent of the second chromosome. Back-crosses showed that a dominant intensifier of Beaded was present in the second chromosome of the Beaded stock. The stock was not homozygous for the intensifier, since in all these experiments there were two classes of results, one in which no linkage was apparent and the other in which the linkage was of various degrees of closeness. The locus of this second-chromosome intensifier is probably quite near to the locus of black (and not, as Dexter supposed, near that of arc). With arc there was freer crossing-over than with any of the other second-chromosome recessives used, while with black there was very little, as shown by the fact that the female back-crosses gave about the same distribution as the male.

RELATION OF BEADED AND BLACK.

From the crosses with black another interesting feature emerged besides the fact of close linkage between black and the dominant intensifier present in the second chromosome of the Beaded stock. In all the F_1 cultures of the crosses between Beaded and black, the proportion of Beaded was surprisingly low—lower in fact than in any other cultures except the crosses to the pink Beaded which had lost an intensifier. The same low percentage of Beaded appeared also in the F_2 and in the back-crosses from the crosses of Beaded to black. Either black itself acts as a dominant inhibition of Beaded, or there is a mutant dominant inhibitor in the black stocks. The analysis was not carried far enough to enable us to distinguish between these two views.

INTENSIFICATION OF BEADED BY VESTIGIAL, AND THE VESTIGIAL ALLELOMORPH IN BEADED.

Dexter found that when Beaded was crossed to vestigial, strap, or antlered, there was from 60 to 90 per cent of Beaded in F_1. These out-crosses were the only ones in which the percentage of Beaded was consistently over 50 per cent. Dexter suggested, and Muller has since proved, that Beaded is like the yellow mouse in that no homozygous Beaded flies are able to survive. For this reason not more than 50 per cent of the flies of an out-cross receive the Beaded gene. That the vestigial, strap, and antlered stocks do not contain Beaded on the scale demanded to explain these F_1 percentages is apparent in every other out-cross of these stocks; for such F_1 flies as well as the F_2 very rarely show anything that would pass as Beaded. The peculiar feature of the stocks vestigial, strap, and antlered is that they are mutant allelomorphs

of a single multiple-allelomorph system. The high percentage of Beadeds in F_1 from the crosses of the above stocks to Beaded shows that the Beaded stock contains still another allelomorph of that system, and that the surplus "Beadeds" in the F_1 cultures were not true Beadeds but were compounds between this new allelomorph and the particular old member used. It is probable that the Beaded stock was homozygous for this allelomorph.

That an allelomorph of vestigial could give precisely this effect was shown by Bridges in his work on "nick," which is an allelomorph of the vestigial system. Pure stock of nick is indistinguishable from a wild stock, but a vestigial-nick compound varies in character from the wild-type through various low grades of beadedness to the very extreme forms. These nick compounds are usually not to be distinguished from true Beaded; except that the extreme range resembles antlered rather more closely than Beaded. 50 per cent or more of the nick compound are antlered-like and the remainder are wild-type.

NO SEX-LINKED MODIFIER.

Dexter found that there was no apparent linkage between Beaded and sex-linked genes, and there was therefore no sex-linked Beaded gene or Beaded modifier present in the Beaded stock.

PARTIAL SEX-LIMITATION OF BEADED.

Beaded had been found by Morgan to be partially sex-limited, i. e., in the stocks and in most crosses a greater percentage of the females showed Beaded than was the case in the males.

MATROCLINOUS EFFECT WITH BEADED.

Dexter discovered a very curious relation, that the sons of Beaded mothers showed a much higher percentage of Beaded than the sons of Beaded fathers. If this were general, as it seemed to be, it would indicate not a sex-linked modifier of beaded, but a precocious action by the Beaded gene upon the cytoplasm of the egg. It may be that a critical stage for the determination of the Beaded character occurs very early in the formation of the egg.

THE POSITION OF THE BEADED LOCUS AS DETERMINED BY STURTEVANT.

Sturtevant found that the locus of Beaded is to the right of that of sooty by a considerable interval.

MULLER'S ANALYSIS OF BEADED—A MORE ACCURATE DETERMINATION OF THE LOCUS OF BEADED.

Muller crossed (June 1915) Beaded from "pure" stock to flies carrying the third-chromosome recessives sepia, spineless, sooty, and rough. Part of the F_1 flies were Beaded and part (the majority) were wild-type. The Beaded F_1 females when back-crossed to the multiple-recessive male gave the normal amounts of recombination and showed that the locus of Beaded was very close to that of rough at the extreme right end of the map as known then. A certain further back-cross (see p. 438, Muller, 1918) showed that the locus of the

dominant Beaded gene was to the right of rough by about 2.7 units. In the above experiment there were 4 rough Beaded recombinations in a total of 153 Beaded flies.

THE LETHAL EFFECT OF HOMOZYGOUS BEADED.

From the Beaded back-cross tests Muller obtained a few flies, as the result of crossing-over, that were both rough and Beaded. One of these flies was out-crossed, and the Beaded F_1's were inbred. In F_2 no rough eyes whatever appeared, although one of the grandparents had been rough. The explanation of this fact is that the F_2 flies that were homozygous for rough were at the same time homozygous for Beaded, and all homozygous Beaded die. Other experiments confirmed this view. The Beaded gene has, like the gene for the yellow mouse, two effects, a dominant somatic effect and a recessive lethal effect. Beaded was not unique in this respect, for certain of the other dominants of *Drosophila,* notably Notch, Streak, and Star, had already been recognized as having a recessive lethal effect.

THE PRESENCE OF CIII IN THE NON-BEADED THIRD CHROMOSOME.

The Beaded gene is present in only one of the two third chromosomes of the Beaded stock flies. When the non-Beaded F_1 flies from the cross of Beaded to the multiple recessives were back-crossed the results (see table on p. 438, Muller, 1918) showed that there was present in the non-beaded chromosome of the stock a gene that cut down the crossing-over in the third chromosome in a characteristic way not distinguishable from the known linkage variation CIII. Other tests showed that this variation gave in combination with the original CIII recombination results similar to those of homozygous CIII. The stock of the originally found CIII was descended from the original Beaded stock, so that it is probable that it was the same linkage variation, CIII, that was present in both. CIII is located near the locus of sooty and eliminated (in heterozygous form) practically all crossing-over to the right of spineless.

THE BALANCING LETHAL—LETHAL-IIIA.

Some of the F_1 non-beaded offspring were mated together to produce F_2 progeny. Analysis of the F_2, and more especially of the constitution of certain recombinations that occurred in F_2, led to the conclusion that the non-beaded, C–bearing third chromosome carried a recessive lethal gene which was distinct from both the Beaded and CIII gene and whose locus was to the right of CIII and to the left of Beaded. Certain later experiments showed that the locus of this lethal was about halfway between ebony and sooty or at 83.

PARALLEL BETWEEN BEADED STOCK AND ŒNOTHERA.

The stock of Beaded apparently bred true, but yet upon out-crossing to unrelated stocks two distinct types occurred in F_1 comparable to the "twin hybrids" of *Œnothera.* There are other features of œnotheras that leave no doubt of the existence of enforced heterozygosis. It is probable that many of the balancing lethals of *Œnothera* are not zygotic lethals of the type just

described, but are gametic lethals that kill either the egg or the pollen. A gametic and a zygotic lethal can form a balancing pair.

ENFORCED HETEROZYGOSIS.

The stock of Beaded continued to breed true for the reason that all homozygous flies died, while the heterozygous type survived. The heterozygous type showed the dominant Beaded character. The heterozygosity was enforced from generation to generation by the presence of the two balancing lethals—the Beaded gene in the one III–chromosome and the lethal-IIIa in the other. The presence of CIII in the lethal-bearing chromosome eliminated crossing-over between the two lethals, so that no gamete free from one or the other could arise by crossing-over.

SPURIOUS MUTATIONS.

It is to be noted that the Beaded stock is constantly heterozygous for three genes Beaded, IIIa and CIII. The presence of CIII prevents crossing-over within a large region of the chromosome, so that whatever mutant genes were situated within this region would likewise be maintained in the same condition from generation to generation. If the mutant gene were in the neighborhood of spineless or to the left of spineless, then in a small proportion of cases there would be crossing-over, and homozygous individuals would arise that would simulate mutations. Thus, if the gene for pink were in the same chromosome with Beaded, a pink IIIa cross-over chromosome would occur about once in 50 times. Such a cross-over gamete fertilizing a pink Beaded egg would give a pink Beaded individual. The ratio would be 49 : 1 and not the familiar Mendelian ratio of 3 : 1, and the result would indeed give the same sort of effect seen in *Œnothera* where certain definite "mutations" recur each generation in a small characteristic per cent of the progeny. In *Drosophila* such ever-sporting strains can be arranged at will, so that the "mutations" will occur in any required proportion. In *Drosophila,* also, double-reciprocal or more complex hybrids may be arranged for, and many interesting and unusual results obtained if desired.

PINK (p).

(Plate 1, Figure 3.)

ORIGIN OF PINK.

The first eye-color, and the character first clearly recognized as a sharp "mutation," in *Drosophila* was "white," found in April 1910, by Morgan. In June 1910, he found a second distinct eye-color mutant "pink." A stock of wild flies that had been set aside was found to contain many flies, both females and males, having eyes of a dark pink color.

DESCRIPTION OF PINK.

When first hatched these flies have a transparent yellowish-pink color which rapidly deepens to ruby. The characteristic color of pink (about day-old flies) is a deep pink with a tinge of magenta. Pink flies more than a week old have this purplish tone quite markedly developed. The old pink flies retain

much of their translucency and, with the light "fleck" or no "fleck" at all, are always in contrast to the more opaque red of the wild-type, with its dark metallic fleck. Pink was found to be completely, easily, and rapidly separated from the wild-type at any age.

INHERITANCE OF PINK.

The pink-eyed flies that were found in the wild stock were bred together and produced only pink offspring. The fact that the pink stock bred true from the first suggested that the mutant character was a recessive. Crosses of pink males to wild females showed this to be true; in F_1 all the flies were of the wild-type. In the first F_2 cultures (Morgan, 1911) considerably less than a quarter of the flies were pink, but this was probably due to the poor methods of culture then available. In the later work pink has hatched in about the expected proportions.

CHROMOSOME OF PINK.

Among the F_2 flies there was no limitation of this pink to the males, as had been the case in the similar experiment with white (Morgan, 1910*a*). Moreover, in the reciprocal cross (pink ♀ × wild ♂), no pink appeared among the F_1 males, as had been the case with white, and the F_2 from this cross gave the same ratio as the direct cross had given, instead of the 1:1 ratio that had appeared among both females and males of the similar cross with white. Morgan interpreted the peculiar method of inheritance of white to mean that the gene for white-eyes was carried in the chromosome that determined sex (Morgan, 1911). The very important conclusion followed that pink was inherited in a manner different from that of white because the gene for pink was in another chromosome from that carrying the gene for white (Morgan, 1911). Soon after this the recessive mutation black body-color was found, and it also was found to be non-sex-linked; that is, its gene was in a chromosome different from the X chromosome. The next step was the discovery (by Sturtevant, February, 1912) that these two mutations black and pink were entirely independent of one another in inheritance, and therefore were considered to be inherited through separate chromosomes. The chromosome carrying the gene for black was thereupon called the "second" chromosome and that carrying pink the "third."

RELATION OF PINK TO WHITE.

With the discovery of pink two distinct eye-colors were on hand, and experiments were started immediately to find the relation between them. In accord with the view then current it was assumed that white was due to "the loss of the color-producer," while pink was due to "the loss of a specific color-determiner." The crosses between white and pink gave wild-type daughters and white sons, which inbred gave in F_2 3 wild-type: 1 pink: 4 white among both males and females. No difference could be discerned between the simple whites and the whites that were genetically pink (double recessives). Pink was referred to as "hypostatic" to white. The more recent view is that the genes for white and pink both belong in the general category of "dilutors" of eye-color and that since white already dilutes the color to the physical limit no further dilution by the pink gene is possible.

RELATION OF PINK TO VERMILION.

A few months after the discovery of pink, Morgan found a third distinct eye-color mutation, "vermilion," which was a sex-linked recessive like white. Because of the prevalent belief in the "presence and absence" view of mutation, an inordinate amount of importance was attached to the relation of vermilion and pink, and enormous numbers of flies were raised in the various crosses to test this relation. Vermilion was conceived as due to the loss of another specific color-determiner; and since the cross of vermilion male to pink female gave only wild-type offspring, it was considered proved that the wild-type was due to the combined action of a pink gene brought in by the pink fly and a vermilion gene brought in by the vermilion parent. The pink eye-color was thus a "disintegration product" due to the loss of the vermilion gene (vP), while vermilion was another disintegration product brought about by the loss of the pink gene (Vp). In F_2 from the cross of vermilion female to pink male, the classes were 3 wild-type : 3 vermilion : 1 pink : 1 "orange." In F_2 there occurred this new eye-color, "orange," which in appearance differed from pink in just about the direction and degree that vermilion differed from the red of the wild-type, or, stated conversely, the "orange" differed from vermilion in the direction and extent that pink differs from the wild-type. This "orange" color was looked upon as the "residium" after the loss of both the vermilion and pink genes. The color shown was ascribed to the action of a gene called the orange gene (vpO), and it was considered that considerable progress had been made in "the analysis of the constitution" of the eye-color of the wild fly. The culmination of this view was reached with the "loss of the orange factor" in the production of the eosin eye-color mutation. It thus became possible to speak of "pure" vermilion, the color produced by the action of the vermilion gene in its absence of all other "determining" genes (except the color "producer" thus: VpoC). Likewise "pure C" flies (vpoC) were the focus of a considerable amount of investigation and speculation.

Systems similar to this were established for body-colors, for wing-lengths, and were on the point of being established for other characters when the whole structure began to collapse. In the first place, it was found that eosin was probably an allelomorph of white, and as this became more and more certain the foundation of presence-and-absence became more and more insecure. Moreover in rapid succession new eye-colors (purple, maroon, cream–a, cream–II, cream–III, sepia, and others) appeared, and each fresh appearance required that the entire edifice be rebuilt. The system finally became so unwieldy that it had to be abandoned in toto. The present simple system, capable of almost indefinite expansion, took its place.

VIABILITY OF PINK—LINKED LETHALS.

In the early work with pink (September 1910), it was observed that the pinks were hatching in smaller proportion than the expected 25 per cent (Morgan, 1911). It is now known that most, if not all, of that apparent inviability was due to the undeveloped state of the culture methods. However, in certain experiments there were sharp differences between the different F_2 cultures that could not be explained on the ground of inviability effects of the usual kind. At the suggestion of Morgan, a graduate student, Miss Moody,

repeated these crosses, and found the same condition present (see Morgan, 1912). Later, Mr. Liff made extensive tests of the effects of various environmental differences (Am. Nat., 1915). He found that the proportion of pinks could be reduced slightly by unfavorable conditions, but not enough to explain the results of Morgan and of Moody. The many cultures raised by Liff were remarkably free from significant deviations from the expected ratios. Five cultures were found that gave similar deviations. By this time the hypothesis of lethals had been developed, and the suggestion was made (by Muller) that these aberrant ratios were to be explained by the presence of an autosomal lethal linked to pink. It now appears that this was probably the true explanation, though the experiments of Liff were not suitable to prove the point. A calculation of the amount of exchange between pink and the lethal showed a recombination per cent of 32.7. This would place the locus of the lethal at about 85 or at about 10, depending on which side of pink the lethal is situated. (See p. 107, for a fuller account of Liff's lethal.) The aberrant ratios found by Morgan and by Moody are also to be explained as the result of a recessive lethal. Roughly, a fourth of the F_2 cultures gave aberrant ratios, while the remaining three-fourths gave normal ratios, as would be expected if the pink grandparent had been heterozygous for a lethal. A calculation of the recombination of pink and this lethal gives a percentage of 7.0, which is a maximum value, since all cultures under 30 flies were omitted from the calculation. The locus of the Morgan-Moody lethal is thus at approximately 41 or 55, and it is certainly a different lethal from the one that was present later in the cultures of Liff. (See p. 64 for a fuller account of lethal-IIIb.)

In the later work upon pink there has been no trouble on account of inviability of pink flies.

DILUTION EFFECTS AND DOMINANCE RELATIONS.

Morgan and Bridges made a study of the dilution effects of pink eye-color in conjunction with the other eye-colors (white, vermilion, and eosin) known at that time (Morgan and Bridges, 1913). Thus it was found that while white, eosin, vermilion, and pink are all strictly recessive as far as the simple heterozygote is concerned, yet for each of these genes it was possible to demonstrate a positive diluting effect in the heterozygote by the addition of suitable further dilution. While a fly of the constitution $\frac{+}{p}$ can not be distinguished from the pure wild, yet flies of the constitution $\frac{w^e \quad v}{w^e \quad v} \; \frac{+}{p}$ or $\frac{w^e \quad v}{w \quad v} \; \frac{+}{p}$ are easily distinguishable from homozygous eosin vermilion (and also from homozygous eosin vermilion pink). Likewise $\frac{w^e \quad +}{w^e \quad v} \; \frac{p}{p}$ was intermediate in color between homozygous eosin pink and homozygous eosin vermilion pink. The diluting power of eosin was greater than that of vermilion or pink, and, as might be expected, the diluting power of white was greatest of all. Thus, while heterozygosity for eosin gave considerable dilution of vermilion pink, white gave a beautiful and much more marked dilution of that same form. Indeed, heterozygosity for white could be detected when merely vermilion was present as a sensitizer $\left(\frac{+ \quad v}{w \quad v}\right.$ slightly more translucent than $\left.\frac{+ \quad v}{+ \quad v}\right)$.

PINK NON-MODIFICATION.

The next eye-color to arise after pink was purple, found by Bridges in February 1912. Purple bore considerable resemblance to pink, and it was thought worth while to test their relation somewhat. The F_1 flies were all wild-type, which shows that the two mutants are non-allelomorphic. In F_2 the ratio approximated 9 wild-type to 7 of a pinkish character. The striking feature of the F_2 was that the double-recessive form purple pink could not be distinguished, except vaguely, from the pinks. An alternative explanation, that purple was in the same chromosome as pink (the third), and because of close linkage no double recessive had really been formed, was shown to be inapplicable as soon as it was found that purple is in the second chromosome. The indistinguishability of the double form from the lighter of the two pinks made the two mutants unsuitable for use together in most experiments. For this reason the curious "non-modification" was lost sight of until other mutations of the "genus pink" (maroon, ruby, garnet, peach, etc.) had arisen, and it seemed to be nearly universal that these various pinks are non-modifiers, or disproportionately slight modifiers, of each other's effects. More exact and extensive tests of these relations are being carried out.

PINK AS THE FIRST BASE OF THE THIRD CHROMOSOME.

A large proportion of the early mutations of *Drosophila* were of various types that do not give easily recognizable Mendelian results, since the ordinary 3 : 1 ratio is not obtained. Explanations for these apparently aberrant cases are now on hand, but when such cases as Beaded, Truncate, "with," Abnormal-abdomen, etc., were encountered early in the work, they tended to obscure the whole situation. Even pink, although a sharp, clearly distinct mutant that did not overlap the original type, at first gave aberrant ratios because of the lethal present. Black was the first autosomal mutant that was recognized as giving orthodox results in every particular, and it was thus natural that black should be chosen as the basis of the second-chromosome. Then pink rose in esteem as soon as it was found that some pink stocks were free from the undesirable feature of poor production in crosses.

At this time black and pink were the most used and best known autosomal mutations. Accordingly, when a search for linkage between autosomal mutants of the type already known between certain sex-linked mutants (yellow, white, vermilion, miniature, and rudimentary) was undertaken, black and pink were the first tested. It was found that there was no linkage between these two mutants (Sturtevant, February 1912), and it was therefore assumed that the genes were carried by separate and distinct pairs of chromosome. Shortly afterwards (March 4, 1912), Bridges found that the new mutant curved was linked to black. Since this was the first autosomal linkage observed, the chromosome carrying the gene for black was defined as the "second chromosome" and the chromosome carrying the gene for pink was defined as the "third chromosome." The "first-chromosome" was of course that already worked out, the sex-determining or X–chromosome. A conference was then held at which a general systematic testing of the linkage relations of all the autosomal mutants thus far on hand was planned. The first of these tests to mature was that of the relation

between pink and maroon (Bridges, April 30, 1912). But, unfortunately, the result was indecisive because of the possibility of "non-modification" of pink by maroon. The F_2 from the cross of pink by ebony gave the first indisputable evidence of third-chromosome linkage (Sturtevant, July 15, 1912). In June 1912, Bridges found kidney-eye and showed that it also was linked to pink (July 23, 1912). With little difficulty Bridges secured the double recessive pink kidney, with which he carried out back-cross tests of the amount of recombination of pink and kidney (November 4, 1912). He also made tests of the crossing-over in the male, and concluded that between pink and kidney there was a slight amount of crossing-over in the male. Subsequent tests showed that the apparent recombinations were due on the one hand to a failure of kidney to show in an occasional fly homozygous for it, and on the other to a confusion between the "seam" in the eye due to kidney and a similar "seam" that occurs in a small proportion of normal flies.

Sturtevant was delayed many generations in his attempt to secure the pink ebony double recessive because of the very close linkage between pink and ebony. The back-cross tests (December 5, 1912) showed that there was approximately 5 per cent of recombinations of pink and ebony as against approximately 15 per cent for pink and kidney. It was naturally assumed that kidney was farther away from pink than ebony; but that is not the case. It has since been proved that the pink ebony was difficult to obtain, and gave little recombination in the back-cross tests, because it carried a dominant linkage variation (CIII). Sepia was discovered soon after kidney and its locus was found to be to the left of that of pink.

Gradually there was built up around pink as a base a map which included sepia, pink, spineless, kidney, spread, ebony (and its allemorph sooty), rough, and beaded, in the above order of loci. Muller did a large part of this later work incidental to building up the stocks for his progeny test for crossing-over (Muller, 1916).

The discovery of the exceptionally valuable dominant mutant Dichæte (Bridges, July 3, 1915) led to the entire recasting of the third-chromosome, with Dichæte as the new base. The recombination data involving pink are considered in the various sections in which they are used to show the relation of the loci of different mutations to that of the pink base.

THE LOCUS OF PINK.

On the basis of the entire body of data, the locus of pink is 7.6 units to the right of that of Dichæte, or referred to roughoid as the zero-point of the third chromosome at 48.0.

ALLELOMORPHS OF PINK.

On several occasions pink has been found unexpectedly, but never under circumstances that entirely ruled out the assumption that it was the original pink present through contamination or by injection through the parent stock. It is possible that one or two of these occasions represent a reoccurrence of the pink mutation or the occurrence of a pink allelomorph so nearly identical with pink that it was not distinguishable from pink.

In May 1913, Bridges found a yellowish pink eye-color that proved to be an allelomorph of pink quite easily separable from pink and recessive to it. This

mutation, called "peach" (see p. 82) proved more rapidly separable from the wild-type than is pink, and has consequently almost entirely supplanted pink in experiments involving that locus, in much the same way that eosin has supplanted white, and has been in turn supplanted by apricot.

In April 1916, Bridges found a second allelomorph of pink called "pink^3" (see p. 164) that was also recessive to pink. Pink^3 was of a brilliant pink eye-color, exceptionally translucent and of a tone as light as that of peach, but without the yellowish tone of peach. Pink^3 flies were further characterized by weak legs and a paler body-color. Their relatively high inviability rendered them nearly useless in experiments and finally led to their extinction.

In January 1917, Mildred Hoge Richards found a third recessive allelomorph of pink, namely "rose" (Biol. Bull., Ict., 1918). Rose (renamed pink^4) is practically identical in appearance with peach in young flies, but does not become as dark with increased age, as does peach. The peach-rose compound is an intermediate.

EVALUATION OF PINK.

The character pink was used very extensively in the early experiments and was on this account made the base of reference of the third chromosome. At present pink has been replaced in use by the more rapidly classifiable allelomorph peach. Also, the base of reference of the third chromosome has shifted to the locus of the dominant mutant Dichæte.

LETHAL–IIIA (WITH CIII AND OPPOSITE BEADED).

The selection for more extreme types of Beaded, carried out by Morgan (see p. 37), resulted in a Beaded stock that apparently bred true to Beaded (December 1911). The analysis by Dexter and especially by Muller (November 1915) showed that Beaded is a dominant that is lethal when homozygous, and that the above stock bred true because of the presence of a balancing lethal in the opposite chromosome. This recessive lethal, now called lethal–IIIa, was kept from escaping from this opposite chromosome by the fact that this chromosome already carried CIII, which prevents practically all crossing-over between the lethal and the Beaded loci. Muller secured lethal–IIIa free from CIII by the occurrence of a rare cross-over, and tests for the position of the gene showed that its locus is midway between ebony and rough or about 83.0. This location is based on the fact that of 19 tested flies that were known to be cross-overs between sooty and Beaded, 10 represented crossing-over between sooty and IIIa, while 9 represented crossing-over between IIIa and Beaded (Muller, 1918, p. 448). The CIII IIIa combination is still much used, especially in balancing other lethal stocks, such as Hairless and Delta.

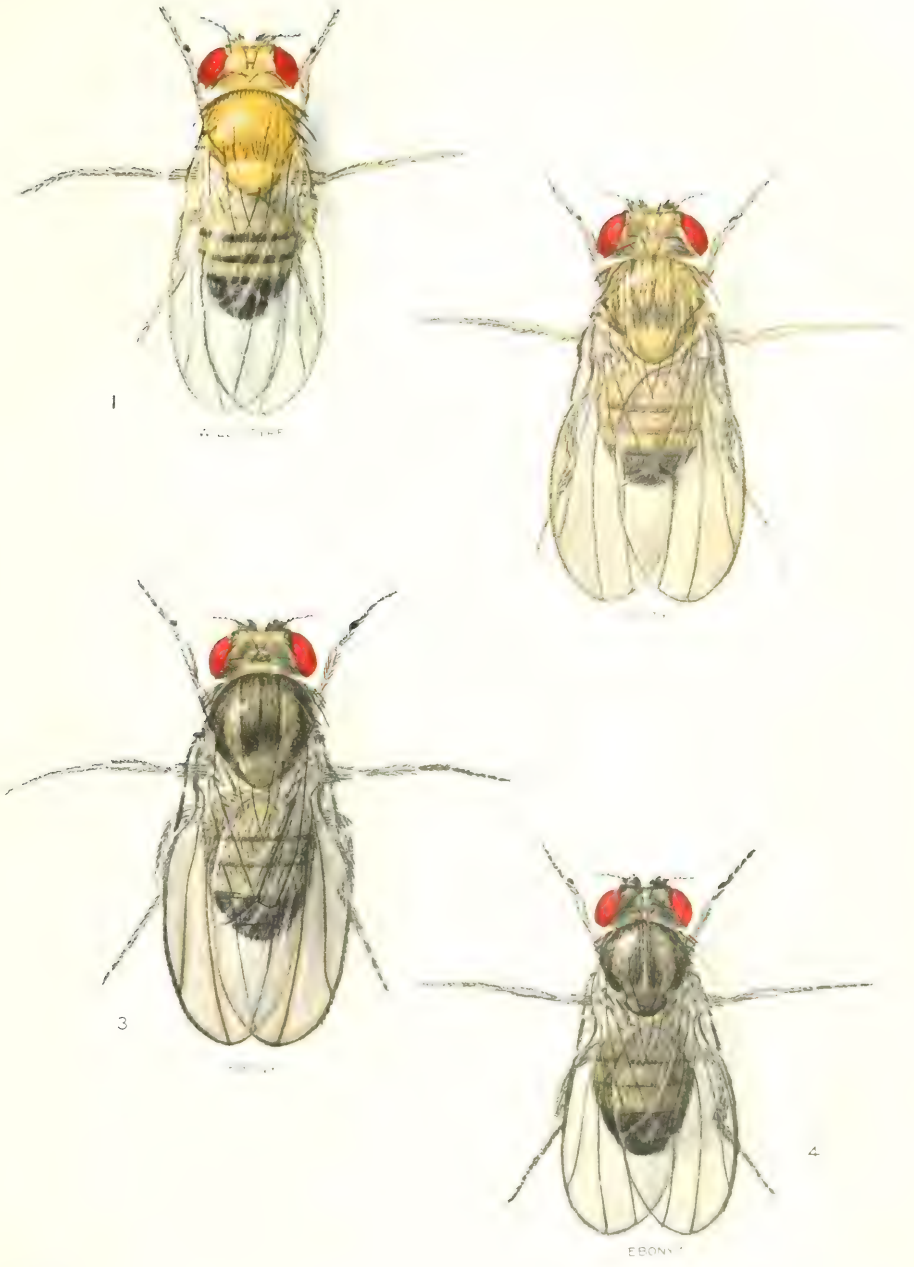
EBONY (e).*

(Plate 2, figure 3; plate 1, figures 7 and 8.)

ORIGIN OF EBONY.

In making up a fresh stock of the mutant balloon-wing, Miss E. M. Wallace noticed that there were present a few flies with a very dark body-color

* See Science, June 27, 1913, for a preliminary account of the inheritance of ebony.



E. M. Wallace pinx.

THIRD-CHROMOSOME BODY-COLORS OF DROSOPHILA

(February 15, 1912). It was supposed that this character was the well-known mutant black.

Balloon was one of the mutants assigned to Sturtevant in the plan for clearing up the linkage relations of the then known autosomal mutants. The presence of "black" in the balloon stock furnished a double recessive with which a back-cross test could be carried out immediately. A balloon "black" male was crossed to a wild and both F_2 and back-cross cultures were raised. The F_2 ratio was 9:3:3:1 and the back-cross ratio was 1:1:1:1 (May 10, 1912). These results showed that balloon and "black" were not linked, and were presumably in separate chromosomes. But during the first counts of the above cultures it was recognized that the "black" was darker and more shiny than normal black, and while very young was slightly more greenish in color. The "black" was crossed to true black, and the F_1 flies, though somewhat darker than wild-type, were by no means as dark as either black or the new "black." Evidently the two characters were not even allelomorphic. The new "black" was finally named ebony.

CHROMOSOME OF EBONY.

Sturtevant then crossed ebony to pink and to vestigial as representatives of the third- and second-chromosomes. The F_2 from vestigial by ebony gave

TABLE 9.—*P_1, vestigial ♀ × ebony ♂: F_2, F_1+♀ and F_1+♂.*

July 15, 1912.	+	vg	e	vg e
Total.....	268	94	79	24

a 9:3:3:1 ratio (table 9); and the F_2 from pink by ebony gave a 2:1:1:0 ratio (table 10). Ebony was also found to give 9:3:3:1 ratios with two other

TABLE 10.—*P_1, pink ♀ × ebony ♂: F_2, F_1+♀ and F_1+♂.*

July 15, 1912.	+	p	e	p e
Total.....	3764	1369	1112	0

second-chromosome recessives, viz., curved and jaunty. These results were definite and proved that the locus of ebony is in the third chromosome.

LINKAGE OF PINK AND EBONY.

The F_2 from pink by ebony was carried out on a very large scale, for the purpose of obtaining double recessives with which to make back-cross tests. No double recessive flies appeared, and this was taken to mean that the loci of pink and ebony are very close together in the third-chromosome. The non-appearance of the double recessive was, of course, due to the lack of crossing-over in the male, and would have been the result, whatever the amount of

crossing-over in the female might be. This was not known at the time; but later Sturtevant mated F_2 pink by F_2 ebony, a procedure that has become a standard method for obtaining double recessives of linked autosomal characters. It took several generations before pink ebony was obtained even by this method (November 21, 1912). The difficulty in obtaining the double recessive was an indication that there was little crossing-over between pink and ebony.

This was confirmed by the results of the back-cross test of heterozygous females (table 11). The usual value for pink ebony recombination was shown

TABLE 11.—P_1, *pink ebony* ♂ × *wild* ♀: *B. C.*, F_1 + ♀ × *p e* ♂.

Dec. 5, 1912.	p e	+	p	e
Total.....	691	880	86	65

to be under 2 per cent. But there was great variation in the ratios. Some of the cultures gave around 25 per cent of recombination. It is now known that the low value was due to heterozygosis for a dominant crossing-over reducer (CIII) that was carried by the ebony chromosome and retained in the pink ebony chromosome. The values around 25 were probably due to homozygosis for CIII, one CIII being present in the ebony chromosome and the other introduced from Beaded. The standard pink ebony value, when no known crossing-over variation is present, is about 24 per cent.

A "repulsion" back cross test of females was also carried out (table 12).

TABLE 12.—P_1, *pink* ♀ × *ebony* ♂: *B. C.*, F_1 + ♀ × *p e* ♂.

Dec. 5, 1912.	p	e	p e	+
Total.....	601	584	4	3

There were very few recombinations, less than 1 per cent. The ebony carried CIII, and the pink was free from CIII.

Considerable difficulty was met with in carrying out back-cross tests of males, because ebony females and pink ebony females were often sterile, though the males were normally fertile. This difficulty disappeared later, and it seems probable that it was due to a special gene in the balloon and ebony stocks. The male back-cross tests gave no cross-overs.

ALLELOMORPHS OF EBONY.

Ebony itself was not used much, except in working out the linkage variation CIII, but in October 1913, Sturtevant found sooty, an allelomorph of ebony that is free from CIII, and this mutant has been very extensively used. Later Sturtevant found two other allelomorphs of ebony, namely, "ebony^3" and "ebony^4." Ebony^4 stock is likewise free from CIII. Full accounts of these allelomorphs are given in separate sections of this publication.

LOCUS OF EBONY.

The bulk of the data on the location of ebony has been obtained with the allelomorph sooty. In table 2 is given a summary of the normal data bearing on the common locus of all these allelomorphs, which is at 70.7.

EVALUATION OF EBONY.

The character ebony is the darkest body-color in *Drosophila melanogaster*, and is in striking contrast to the wild-type. Separations are therefore complete and exceptionally easy. The viability of ebony seems much inferior to that of the wild-type, which is in agreement with the large departure of the character from the wild-type. The allelomorph sooty, that differs from the wild-type much less, is normal in viability. Besides the ebony allelomorphs, there are no other body-color mutants in the third chromosome, so that no useful characters are masked by this color. The presence of CIII, prevents the use of ebony in determining normal recombination percentages. The other allelomorphs of ebony, especially sooty, so far surpass ebony in usefulness that ebony itself has only been used in the early work of determining the chromosome of autosomal mutants and in working out the effect of CIII.

The ebony locus has been one of the three or four most valuable loci known in the third chromosome. It is situated in the "right" limb of the chromosome, about 12 units to the right of spineless and 20 units to the left of rough. More recently the exceptionally valuable dominant mutant Hairless has appeared in this same region, the locus of Hairless being only 1.2 units to the left of ebony. These two loci are used interchangeably, in much the same way as are forked and Bar in the X–chromosome, and in calculating recombination per cents the data can be converted from one type to the other through knowledge of the distance between the loci.

MAROON (ma).

(Plate 1, Figure 10.)

ORIGIN OF MAROON.

The eye-color mutation now called "maroon" was first found by Bridges (March 13, 1912) in a culture of a stock of wild flies that had been brought from Kushla, Alabama, during the previous summer. Both females and males were found, which indicated that the mutation was probably not sex-linked; for sex-linked characters are usually found as males only, or with the males predominating. Sex-linked dominants could appear as readily in females as in males; but the fact that pairs and mass cultures of the new color bred true to the color and never threw red showed that the color was recessive.

RELATION OF MAROON TO PURPLE.

This eye-color was considered to be the same as "purple," which is a second-chromosome eye-color that had been found only about a month before (February 20, 1912). That it was not purple was proved when crosses (10 pair-matings) between purple and the new color were carried out, for the F_1 flies were in every case red. This result is always obtained when two non-allelomorphic recessive eye-colors are crossed together, since each is dominated

by its own wild-type allelomorph brought in by the other parent. The fact that two eye-colors almost identical in appearance were genetically independent aroused considerable interest, since it was the first case in which we had found that the same somatic effect was producible by each of two distinct genes. Such cases of "mimics" have since been found to be frequent.

DESCRIPTION OF MAROON.

The eye-color is fairly well described by its name "maroon," since it is a deep purplish pink like old red wine. Perhaps the names crimson or carmine would have fitted as well. The color is slightly flocculent, and translucent rather than transparent. Maroon resembles purple closely, but has been found to differ from purple by having a less pronounced purple tone, by being more flocculent, and by becoming darker with age, old maroons acquiring a sepia color like that of old pinks. The difference between the maroon and wild-type characters becomes progressively less with the age of the culture.

THE RELATION OF MAROON TO PINK—NON-MODIFICATION.

The fact that maroon was genetically distinct from purple led to its testing with pink, which is another quite similar eye-color that had been found two years previously (Morgan, 1911). Pink is lighter in color, more transparent, and lacks all purplish tone, so that it should be separable from maroon in the majority of cases, probably with more than 85 per cent of completeness. Crosses

TABLE 13.—P_1, *maroon* ♀×*pink* ♂; F_2, F_1+♀×F_1+♂.

Apr. 30, 1912.	+	p	ma	p ma
B 19.....	220	87	98	.....
B 20	265	96	117	.....
Total....	485	183	215	.....

of maroon by pink (3 pair-matings each way) gave wild-type F_1 flies, showing that the two were not allelomorphic. Two mass cultures of these F_1 wild-type flies were inbred in hopes of getting the double recessive pink maroon in F_2. The F_2 flies were examined very carefully and separately as completely as possible. It was expected that a quarter (or less, if maroon were in the third chromosome with pink) of the flies would be of a color as much lighter than pink as maroon is lighter than the wild-type. No such light flies were found (table 13).

Up to this time pink was the only mutant known to be in the third chromosome. It was not certain from the result of this pink maroon F_2 that maroon is in the third-chromosome, since the alternative hypothesis that certain classes were not separable was also possible.

That the double recessive pink maroon is no lighter than pink would indicate that these two mutants are "non-modifiers" of each other (see p. 118). Subsequent work has indeed shown that "non-modification" is the usual relation between the different "pinks" that have arisen.

CHROMOSOME OF MAROON.

To determine whether the apparent absence of pink maroons was real and due to linkage, further tests of the linkage relations of maroon were made. Maroon was crossed to arc, which is a second-chromosome recessive. The F_2 gave plenty of arc maroons, so that it was known that maroon was either in the third-chromosome or else in the second-chromosome a long distance away from the locus of arc (table 14).

TABLE 14.—P_1, *arc* ♀ × *maroon* ♂; F_2, F_1 + ♀ × F_1 + ♂.

July 13, 1912.	+	a	ma	a ma
350.....	158	54	41	20

The presence of the arc maroon double in F_2 would now (since the discovery of "no crossing-over in the male") be accepted as complete proof that maroon is not in the second-chromosome; but at that time the only method of proof recognized was to show by relatively large numbers that the ratios were actually those of independent assortment. As the back-cross was recognized as being more efficient than an F_2 it was decided to obtain these larger numbers by arc maroon back-crosses. Back-cross tests of the male and of the female

TABLE 15A.—P_1, *arc maroon* ♀ × *wild* ♂; *B. C.*, F_1 + ♀ × *a ma* ♂.

Nov. 2, 1912.	a ma	+	a	ma
C 58.....	98	131	94	108
C 62.....	85	81	83	73
Total....	183	212	177	181

TABLE 15B.—F_1 + ♂ × *a ma* ♀.

C 63a.....	80	121	92	145
C 63b.....	61	103	85	95
C 64.....	96	89	66	77
Total....	237	313	317	243

were both undertaken, and these gave identical results, namely, no observable linkage. The recombination classes totaled 918 out of 1863, or 49.3 per cent, where 50.0 is expected on independent assortment (tables 15A and 15B). These results proved that maroon is not in the second-chromosome, for it had meanwhile been demonstrated (Morgan, 1912) that there is no crossing-over of the second-chromosome in the male.

That maroon is in the third-chromosome was proved directly by the F_2 of the cross of maroon by ebony. Ebony is a recessive dark body-color mutation that had just been shown (May 1912) by means of a pink ebony F_2 to be in

the third chromosome (Sturtevant, 1913). The F_2 from the maroon by ebony cross gave a 2 : 1 : 1 : 0 ratio, which is the result characteristic of no crossing-over in the male (table 16). The F_2 from the maroon by pink cross was thus

TABLE 16.—P_1, *maroon* ♀ × *ebony* ♂: F_2, F_1 + ♀ × F_1 + ♂.

Nov. 21, 1912.	+	ma	e	ma e
C 156.....	269	94	80	.....
C 157.....	230	94	62	.....
Total....	499	188	142	.....

proved to have been a 2 : 1 : 1 : 0 linkage ratio, and was in fact the first such result obtained for the third chromosome.

EARLY TESTS OF THE LOCUS OF MAROON BY EBONY AND BAND.

An attempt was made to secure the double recessive maroon ebony, with which to conduct a back-cross test of the amount of recombination of maroon and ebony. Repeated crossing of F_2 maroons by F_2 ebonies gave no maroons or ebonies in F_3, hence none of the flies tested had contained cross-over chromosomes. From this it was concluded that maroon is probably quite close to ebony in the chromosome. Afterwards it became clear that the absence of such cross-overs was due to a cross-over variation (CIII) which was present in the ebony stock and which reduces crossing-over, rather than to nearness of the maroon and ebony loci.

TABLE 17.—P_1, *maroon band* ♂ × *wild* ♀: *B. C.*, F_1 + ♀ × *ma bn* ♂.

Dec. 2, 1913.	ma bn	+	ma	bn
III-47.....	67	119	31	18

The next attempt at locating maroon was by means of "band," a recessive thorax pattern, that had been shown to be in the third-chromosome by the result of the pink band F_2 (see p. 79). The locus of band was itself not yet known, but the maroon band value would furnish a needed link in the location of both maroon and band. The maroon × band F_2 culture gave 144 wild-type : 73 maroon : 72 band : 0 maroon band, which is only 1 fly out from a perfect 2 : 1 : 1 : 0 ratio. By mating the F_2 maroons and bands to each other, some maroon off-spring were obtained in F_3, which, mated together, gave maroon band in F_4. Only one back-cross culture was raised, and this gave 21.9 per cent of recombination (table 17).

In the summer of 1914 an attempt was made to repeat and amplify the maroon band recombination test, but it was found that in some unexplained manner the stock was now undoubtedly not maroon band but was pink band.

LINKAGE RELATIONS OF MAROON TO DICHÆTE AND HAIRLESS IN HETEROZYGOUS CHI.

Little more was done with maroon until the discovery and location of two excellent third-chromosome dominants "Dichæte" and "Hairless" had made possible direct back-cross tests bearing on the locus of maroon. The original simple maroon stock had meanwhile been thrown away, and the only stock of maroon kept was that containing dwarf. (For the origin of this stock see p. 101). Accordingly, the maroon dwarf × Dichæte Hairless back-cross gave additional information as to the location of dwarf. The locus of Dichæte was quite near the left end of the third-chromosome as then known, while that of Hairless was near the middle. A maroon dwarf male was out-crossed to a heterozygous female carrying both these dominants in the same third-chromosome, and the F_1 Dichæte Hairless females were tested by mating to maroon dwarf males. The result was surprising, for instead of about 25.0 per cent of recombination as expected for Dichæte and Hairless, only 6.1 per cent was observed (table 18). Nearly all of this recombination (5.9) was due to

TABLE 18.—P_1, *Dichæte Hairless* ♀ × *maroon dwarf* (CHI) ♂: *B. C.*, F_1 *D H* ♀ × *ma dw* ♂.

July 15, 1917.	D H	ma dw	D ma dw	H	D dw	ma H
7,377	117	80	3	9	...	...
7,450	151	110	3	8	...	...
7,451	85	64	3	3	...	...
7,456	36	36	2	4	...	1
7,540	115	89	12	8	...	1
Total	504	379	23	32	...	2

crossing-over between Dichæte and maroon, with only a very little (0.2) between maroon and dwarf, and none at all between dwarf and Hairless. This situation was at once seen to be a parallel to that described by Sturtevant and by Muller for crossing-over in flies heterozygous for the cross-over variation CHI. It had been found that in heterozygous CHI there is only about 2 to 5 per cent of recombination of pink and ebony, and that most of this is in the region very close to pink. There is usually about 23 per cent of recombination of pink and sooty. The Dichæte Hairless stock was known to be free from CHI, and this variation must therefore have been introduced through the maroon dwarf stock. Such proved to have been the case; the maroon dwarf flies were found to be homozygous for CHI. There is no known connection between the maroon dwarf stock and any other stock containing CHI, but it is possible that there is such a connection. If so, we are required to assume that the CHI mutation occurred very early in the *Drosophila* work before the occurrence of the Beaded mutation (May 1910), and that the ebony mutation took place in the chromosome already carrying CHI. Further, that this CHI–chromosome became distributed very widely by crosses of Beaded, and of Truncate and others of the many mutations that arose in the Beaded stock. Finally, the mutation maroon, and later still the mutation dwarf, arose in such a CHI–chromosome.

The occurrence of two maroon not-dwarf Hairless individuals in the cross of table 18 proved that the locus of dwarf is to the right of that of maroon, and also that the locus of maroon is between Dichæte and Hairless (that is, not to the right of Hairless). Because of the peculiar distribution of the effects of Cıı the locus of maroon could not, from these data, be determined more closely than that it is somewhere between Hairless and a limiting point about 5 units to the right of Dichæte.

LINKAGE RELATIONS OF DICHÆTE MAROON AND DWARF IN HOMOZYGOUS CIII.

A way out of this uncertainty was offered by testing the amount of recombination in the presence of homozygous Cıı, under which condition the amount of crossing-over throughout the part of the chromosome from pink

TABLE 19.—P_1, *maroon dwarf* (*C*III) ♂×*Dichæte* (*C*III) ♀; *B. C.*, F_1 *D* ♀×*ma dw* ♂.

Sept. 24, 1917.	D	ma dw	D ma dw	+
7,652	123	123	8	14
7,763	156	77	4	12
7,764	110	35	3	11
7,765	89	57	4	7
7,766	69	37	1	3
7,767	126	42	4	16
7,925	154	75	3	9
7,926	141	61	2	7
7,927	62	24	1	4
8,048	52	47	5	..
Total	1082	578	35	83

to ebony is raised to or above normal. A maroon dwarf Cıı male was outcrossed to a female carrying Dichæte and Cıı in the same chromosome. The F_1 Dichæte females were then back-crossed by maroon dwarf Cıı males (table 19). There was 6.6 per cent of recombination of Dichæte and maroon, which is practically the same as that from the heterozygous–Cıı experiments. This result proved that maroon was really not far from the left-most of the positions indicated as possible by the previous experiment. Had maroon been really farther to the right of Dichæte than about 7 units, the increase of crossing-over due to homozygous Cıı would have given a Dichæte maroon value greater than that observed.

In spite of the freer crossing-over due to homozygous Cıı no recombinations for maroon and dwarf were observed among the 1,778 flies. Accordingly, dwarf is very close indeed to maroon.

LINKAGE RELATIONS OF MAROON TO DICHÆTE AND HAIRLESS IN THE ABSENCE OF CIII.

The occurrence of two maroon Hairless flies in the maroon dwarf × Dichæte Hairless back-cross (table 18) gave an opportunity for again studying the

normal linkage relations of maroon. CHH is located somewhere to the right of Hairless, so that crossing-over between maroon and dwarf gave maroon Hairless gametes free from CHH. Since the father was homozygous for maroon, dwarf, and CHH, the maroon Hairless zygote from such a cross-over gamete is heterozygous for CHH. One of the two recombinations found was a male, and this fact gave a good means of picking out in the next generation progeny free from CHH. Thus, because of no crossing-over in the male, only two kinds of gametes are formed (ma H and ma dw CHH) and the offspring that show

TABLE 20.—*Dichæte × maroon Hairless; B. C., F_1 D H ♀ × ma H ♂.*

Oct. 17, 1917.	D H	ma H	D ma H	H	D	+	ma	D ma
7,819.....	55	63	3	2	33	2	10	.....
7,820.....	86	119	3	4	56	3	27	.....
7,828.....	108	91	5	2	57	4	20	1
Total...	249	273	11	8	146	9	57	1

the character Hairless are known to be from the ma H gametes and to be free from CHH. The male was accordingly out-crossed to a Dichæte female and the Dichæte Hairless offspring were of the desired composition $\frac{D}{+}\ \frac{+}{ma}\ \frac{+}{H}$. While females of this constitution might have been tested directly by maroon dwarf CHH males, it was thought best to secure some uncomplicated maroon males. This was done by mating together the Dichæte Hairless males and

TABLE 21.—*P_1, Dichæte × maroon Hairless; B. C., F_1 D H ♀ × ma ♂.*

Oct. 30, 1917.	D	ma H	D ma H	+	D H	ma	D ma	H
7,978.....	70	80	6	5	20	33	1	.....
7,979.....	53	59	2	3	10	9	1	1
7,984.....	77	85	3	2	14	20	3	1
8,007.....	93	73	3	1	18	15	1	2
Total....	293	297	14	11	62	77	6	4

females. Crossing-over between maroon and Hairless gave a few flies of the composition $\frac{+}{+}\ \frac{ma}{ma}\ \frac{+}{H}$. These mated together gave 52 maroon Hairless to 27 maroon offspring (culture 7813), which is the 2:1 ratio expected from the lethal nature of homozygous Hairless. A continuous supply of the proper heterozygotes was maintained by taking advantage of the balanced lethal nature of the combination $\left(\frac{D}{+}\ \frac{+}{ma}\ \frac{+}{H}\right)$. All the homozygous Dichæte and all the homozygous Hairless from the mating of two such flies are killed off, so that all the Dichæte Hairless offspring are known to be only heterozygous

for these two genes, and except for a few cross-overs, are of the parental constitution $\left(\frac{D}{+}\ \frac{-}{ma}\ \frac{+}{H}\right)$.

While waiting for the maroons to hatch, a few of the heterozygous females were mated to maroon Hairless brothers. The results give among the Hairless offspring a measure of the Dichæte maroon recombination (3.5) and among the not-hairless flies a three-point back-cross distribution (table 20). When these latter results are combined with those from the regular back-cross (table 21) there is a total of 977 flies of which 736 are original combinations, 34 simple recombinations for Dichæte and maroon, 196 simple recombinations for maroon and Hairless, and 11 double recombinations, which are due to crossing-over in both regions at once. The position of maroon as found from the above data is about 4.2 units to the right of Dichæte, and there was 21.2 per cent of recombination for maroon and Hairless.

REAPPEARANCES OF MAROON.

During the six months following the discovery of purple (March–August 1912), there were 13 recorded appearances of "purple" eye-colors. Of these new "purples," the first (purple 1, March 13, 1912) proved to be not purple, but a very closely similar eye-color which was renamed "maroon." The tests carried out with the other "purples" showed that purples 5, 6, and 13 were

TABLE 22.—*P_1 speck "Color" (=maroon) ♂ × wild ♀; B. C., F_1 + ♀ × sp ma ♂.*

Feb. 23, 1914.	sp ma	+	sp	ma
66.....	59	54	55	52

also maroon. Purples 5 and 6 were shown to have come from closely related stocks, so that it is probable that they had a common origin. No such connection could be traced between purple 1 (maroon), and these, so that it is highly probable that there were two separate occurrences of the maroon mutation. The pedigree in the case of the other early appearance of maroon (pr. 13) is not clear.

There have been two more recent appearances of maroon that seem to be entirely independent and to have been due to reoccurrences of the mutative process. For determining the locus of the second-chromosome recessive "arc," the double recessive arc speck was obtained in F_4 from the cross of arc by speck. The mating of F_2 arc by F_2 speck flies produced among the F_3 flies, 3 strikingly small females with purplish eyes (November 12, 1913; II, 106). These females were sterile, but from their brothers and sisters more "dwarfs" were obtained, which gave rise to our present stock of the mutation dwarf. The occurrence of speck and the new eye-color in the same F_3 flies gave an opportunity for making at once a back-cross test for speck and "color." Such a test was made and gave 107 recombinations in a total of 220 flies, or 48.7 per cent of recombination, where 50.0 is expected if the two mutants are in different chromosomes (table 22). This back-cross culture was also interesting,

since it gave rise to the sex-linked mutation "facet" (Morgan and Bridges 1916, p. 761). The F_2 from a cross of "color" to ebony gave a typical 2 : 1 : 1 : 0 ratio, which showed that "color" was in the third-chromosome (table 23).

Before attempting to find the locus of "color" by recombination tests, it was decided to try direct crosses with existing third-chromosome eye-colors, this being the method by which "peach" had been shown to be allelomorphic to pink. Crosses of "color" to pink, to peach, and to sepia gave only wild-type offspring, but crosses between "color" and maroon gave maroon-like offspring. A careful comparison of "color" and maroon was then made, and it was decided that there was no clear difference between them. "Color" is therefore maroon or an allelomorph so similar as to be indistinguishable, unless by special methods, which were not applied.

That there had been a reoccurrence of the maroon mutation is certain both from the pedigree and from the fact that the new maroon was at the same time

TABLE 23.—P_1, *"Color"* (=*maroon*) ♀×*ebony* ♂; F_2, F_1+♀×F_1+♂.

Feb. 17. 1914.	+	ma	e	ma e
64.....	84	58	60	0
65.....	142	55	57	0
Total....	226	113	117	0

dwarf and CIII, which are closely linked to maroon and which were not present in the original maroon.

In December 1914, a culture (880), which was part of some experiments on non-disjunction, gave about a quarter of the offspring of a "maroon-like" color, and also a diluted eosin, which from its similarity to eosin purple, was taken to be the double recessive eosin "maroon-like." In this case the first step taken was to cross the new color to the other known eye-colors. With purple and pink the color gave red offspring, but with maroon it gave maroon-like offspring. The color seemed to be identical with maroon (not-dwarf) and so far as could be traced is of entirely independent pedigree. No stocks of the previous maroon were on hand at that period except the one which contained at the same time dwarf and CIII, and since maroon-880 did not contain dwarf or CIII, it is practically certain from this evidence alone that the new maroon was of fresh origin.

It is thus probable that the mutative process responsible for maroon has occurred on at least four separate occasions. This property of the recurrence of the same mutation has since been found to be characteristic of several other loci of which, vermilion, rudimentary, and cut are the most striking examples.

THE ORDER OF THE LOCI PINK AND MAROON, AS SHOWN BY PARALLEL BACK-CROSSES.

The position of maroon, as indicated by the total of the Dichæte maroon recombination data was 4.2 units to the right of Dichæte. But the Dichæte

pink recombination per cent was almost identical (namely, 5±), and since there was considerable variation from experiment to experiment in both these

TABLE 24.—P_1, *maroon*×*Dichæte; B. C.*, F_1 *D* ♀×*ma* ♂.

Oct. 30, 1920.	D	ma	D ma	+
12,071	95	88	11	12
12,072	118	96	12	14
12,073	113	129	13	15
Total	326	313	36	41

determinations, the position of maroon with reference to pink was uncertain, although the indication was that maroon is slightly to the left of pink, that is, not so far distant from Dichæte as pink is. Direct tests of the percentage

TABLE 25.—P_1, *Dichæte maroon*×*pink Hairless; B. C.*, F_1 *D H* ♀×*p* ♂.

Nov. 28, 1920.	D	p H	D p H	+	D H	p	D p	H
12,188	32	30	7	3	12	5	1	2
12,189	167	151	2	8	36	43	4	4
12,190	135	142	6	12	38	43	3	4
12,213	114	111	3	8	39	33	..	3
Total	448	434	18	31	125	124	8	13

of recombination of pink and maroon were impossible in the absence of a double recessive, and in view of the probability that the double recessive would be indistinguishable from pink.

TABLE 26.—P_1, *Dichæte maroon*×*pink Hairless; B. C.*, F_1 *D H* ♀×*ma* ♂.

Nov. 28, 1911.	D ma	H	D	H ma	D ma H	+	D	ma H
12,186	86	85	8	11	24	38	4	4
12,187	80	87	5	10	22	33	1	2
12,195	73	61	3	6	21	28	2	1
12,197	77	91	6	4	22	33	2	1
12,209	57	63	2	2	18	21	..	2
12,214	67	71	10	8	21	16	1	3
Total	440	458	34	41	128	169	10	13

The method of parallel back-crosses had been devised to show the probable order of loci in cases where direct tests offer difficulties. In the present case it was desired to compare the Dichæte maroon recombination per cent with the Dichæte pink recombination per cent under like conditions as far as linkage

variations were concerned. Flies heterozygous for the three loci, Dichæte, pink, and maroon, were secured by mating Dichæte maroon flies (from table 24) to pink Hairless. Some of the F_1 Dichæte Hairless flies were tested by back-crossing to pink, giving a Dichæte pink recombination per cent, while others were tested by back-crossing to maroon, giving a Dichæte maroon recombination per cent. These two per cents are comparable, for whatever linkage variation is present in one lot, should also be present in the other sample of sister flies, and the only disturbing differences should be those involved in random sampling. The Dichæte pink recombination per cent is 5.8 (table 25) and the Dichæte maroon per cent is 7.6 (table 26). The fact that the Dichæte maroon per cent is the larger indicates that the locus of maroon is to the right of that of pink. The number of sisters tested was not enough to make the indication certainty.

SYNTHESIS OF SCARLET DEFORMED PINK MAROON STOCK.

While the previous experiment did not make certain that the locus of maroon is to the right of that of pink, it made that order more probable than

TABLE 27.—*P_1, scarlet Deformed pink × maroon: B. C., F_1 Df ♀ × ma ♂.*

Mar. 9, 1921.	Df	Df ma	ma	+
12,550.....	91	4	106	16
12,551.....	112	2	103	9
12,552.....	74	2	104	32
12,553.....	96	3	126	12
Total....	373	11	439	69

the reverse; and consequently further work was started on the assumption that the order pink maroon is correct. This further work was the synthesis of a stock containing the dominant Deformed and the recessives scarlet pink and maroon, to be used in locating more accurately some of the mutants very near pink. The multiple scarlet Deformed pink had been obtained (see p. 96), and it was mated to maroon. The F_1 Deformed heterozygotes were then mated to maroon. If the locus of maroon is farther to the right of Deformed than pink is, then some proportion of the back-cross flies that are Deformed maroon will have resulted from crossing-over between pink and maroon and will be st Df p ma in one chromosome and simply maroon in the other. Accordingly, Deformed maroon flies from the back-cross (table 27) were tested for the presence of pink by outcrossing to pink. Four such individuals gave only red-eyed flies, but one gave Deformed pink-eyed flies as half the total. These Deformed pink flies were known to be of the desired constitution, namely, st Df p ma in one chromosome. They were mated to ma dw CIII males. The resulting Df ma flies formed a balanced stock, in which crossing-over was nearly eliminated by CIII, in which the sterility of dwarf females prevented the accumulation of ma dw CIII flies and in which the homozygous Deformed eliminated homozygous st Df p ma zygotes. This balance is not perfect, be-

cause C[III] allows some crossing-over; but, with some watching, the stock has maintained itself. The emergence of the st Df p ma chromosome furnished the final proof of the order of the loci pink and maroon. Among the 384 Deformed flies of table 27 there were 11 that were maroon, which corresponds to 2.9 per cent of recombination. This datum, in combination with the information from the other crosses involving maroon, places the locus of maroon at about 2.7 units to the right of pink, or at 50.7.

EVALUATION OF MAROON.

Maroon is satisfactory in all respects except that of ease of classification. As the cultures become older the maroons that hatch are more and more dense in pigmentation and correspondingly harder to distinguish with certainty from the wild-type. The addition of fresh food retards this changing-over as is the case with Abnormal, but our present culture methods make such addition a special task.* The locus of maroon is better than that of pink in one respect, namely, that it is nearer to the locus of spineless, and covers that gap better; but for general work pink is to be preferred.

LETHAL-III B—LETHAL WITH PINK.

In some of the early work with pink (September 1910) it was observed that the pinks were hatching in smaller proportion than the expected 25 per cent (Morgan, 1911). It is now known that most, if not all, of that apparent non-viability was due to the undeveloped state of the culture methods. However, in certain experiments there were sharp differences between the different F_2 cultures that could not be explained on the ground of uniform inviability effect. Most of the early breeding was done by means of mass-cultures, and it was thought that some error (such, for example, as non-virginity) had resulted in an admixture of flies that could not be expected to produce the usual ratio. For this reason Miss Moody repeated the crosses, paying strict attention to all the details of culture methods. Miss Moody's cultures (raised in March 1912; published by Morgan, 1912), were raised from pair matings, but unfortunately in culture bottles of such small size that the output was reduced to an average of about 35 flies per culture-bottle, while about 250 flies are now obtained. About a quarter of the cultures (32 out of 131) gave no pinks at all, while about a score of other cultures gave a very small proportion of pinks. The remaining cultures gave about the expected ratio of red to pink.

An attempt was made to explain the aberrant ratios on the ground that pink hatches later than the wild-type flies. Mr. Liff (Am. Nat., 1915) made a careful study of this possibility, and found that while there was a slight delay, it was by no means enough to explain the results of Morgan and of Moody.

Among the cultures raised by Liff were five that were extreme departures from expectation, though agreeing with each other. It was suggested by Muller that the deficit in the pink could be explained by assuming that there was an autosomal lethal linked to pink, and that the pinks that did appear were therefore crossovers. The work of Rawls (Biol. Bull., 1913), of Morgan

*It has now been found that the difficulty of the separation of maroon from not-maroon is overcome by making all flies homozygous vermilion.

(Science, 1912) and others had shown the existence of sex-linked lethals, and it was realized that non-sex-linked lethals were to be expected, though much harder to detect or to demonstrate. The more recent work has furnished many instances of such autosomal lethals, and methods have been developed for handling them. (See p. 46 for a further account of Liff's lethal.)

The aberrant pink ratios found by Morgan and by Moody are to be explained as the result of a recessive lethal. The results of Moody are well adapted to show this point, since roughly a quarter of the cultures gave lethal ratios and the remaining three-quarters gave normal ratios, as would be expected among the F_2 cultures in case the P_1 pink was heterozygous for a lethal. Certain of the F_2 cultures in cases that gave no pinks must really have belonged among the normal cultures, since it is to be expected that, with such low numbers of offspring, no pinks should occur in a definite proportion of the cultures, according to the laws of chance. For this reason only cultures producing 30 or more

TABLE 27A.—*Classes of gametes and zygotes produced by wild-type flies heterozygous for pink and for a lethal in the same homolog.*

		Original combinations (n).		Recombinations (x).	
Eggs		p l	+ +	p +	+ l
Sperm	p l	Dies	+	p	Dies
	+ +	+	+	+	+
Total		Wild-type $= 3n + 2x$, $p = x$.			

flies can safely be considered in our calculations (27 red : 0 pink gives a deviation from a 3 : 1 ratio of three times the standard error). The linkage of pink and the lethal was so close that there is little difficulty in picking out from among these latter cultures those that are significantly different from the normal ratio. Such cultures gave a total of 37 pink and 1,538 wild-type, which indicates 7.0 per cent of recombination for pink and the lethal. This is to be considered as a maximum value, since the omission of all cultures having less than 30 flies probably eliminated some lethal, together with the non-lethal cultures. It was calculated by means of the following analysis:

The parents of these cultures were heterozygous for pink and for a third-chromosome recessive lethal carried by the same homolog $\left(\frac{p \quad l}{+ \quad +}\right)$. The eggs of the female were of four kinds (p l, + +, p +, + l), but the sperm of the male were of only two kinds (p l, + +), because of the fact of no crossing-over in the male. As shown by table 27A, the pink flies that hatched were all recombinations (x) while the wild-type flies were a complex class constituted from three original and two recombination classes (3n + 2x).

In the absence of further tests, it is impossible to tell whether the locus of this lethal is to the right or to the left of the locus of pink, i. e., whether it is at 55 or at 41. The locus of the lethal present in a few of Liff's cultures gave 32.7 per cent of recombinations with pink, and it is therefore apparent that

Liff's lethal was not the same as that originally present in the cultures of Morgan and of Moody.

WHITE-OCELLI (wo).

ORIGIN AND DESCRIPTION OF WHITE-OCELLI.

On the dorsal posterior part of the head of *Drosophila* there is a group of three "simple eyes" or "ocelli" (plate 1, fig. 1). Each ocellus is about four times the area of an ommatidium of the compound eye. The ocelli of wild flies seem a dilute brownish-red color, about that of a weak coffee infusion. The ocellus is transparent, and not dark in color, but there is a crescent-shaped deposit of dense brownish-red pigment on the inner side of the two posterior ocelli and on the posterior side of the anterior ocellus (plate 1, fig. 1). The apparent color of the ocellus thus changes with the angle at which it is viewed. Looking directly into the ocellus, one sees only a pale transparent "coffee" color; looking at the ocellus from the side, one sees a deeper coffee color varying in intensity with the degree to which the pigment deposit is seen through the ocellus. In many flies there is considerable dark or black pigment deposited on the whole triangular area occupied by the ocelli and by the associated group of bristles and hairs.

It had been observed that all the flies of the white-eyed stock had white ocelli as well as white eyes. All color is absent from the ocelli, and the deposit of brownish-red pigment is entirely absent. The triangular area of black pigment is more conspicuous by contrast. It was concluded that there was probably a correspondence between the color of the ocelli and of the compound eyes, so that the white ocelli of the white-eyed stock were only another effect of the gene responsible for white eyes.

Soon after this it was found that the ocelli of the vermilion-eyed stock were very pale in color—nearly as pale as those of white-eyed flies. A barely detectable tinge of yellow is present and a suggestion of the pigment deposit. All vermilion stocks examined have this same type of ocellus, even those due to new mutations of the vermilion locus. If, as is very probable, this dilution of the ocelli color of vermilion-eyed flies is likewise an effect of the vermilion gene, then it is disproportionately great in amount. The dilution of the ocelli color in vermilion-eyed flies is much greater than one would expect from the degree of dilution from red shown in the eye-color of vermilion flies.

WHITE-OCELLI IN THE BLACK STOCK.

That the color of the ocelli could vary independently of that of the eyes became apparent when it was found by Bridges that many of the flies of the stock of the mutation black body-color had white ocelli (June 21, 1912). A count showed that 27 of the flies had white-ocelli to 20 that had the ordinary coffee-colored ocelli. A careful comparison of the eye-colors of these two kinds of flies was made, but no other difference could be detected; the eye-color of both was the same as that of the wild fly. Some of the flies with white ocelli were bred together, and at once a pure stock was obtained.

A search was made among the other stocks, and it was found that nearly all of them had ocelli of the wild-type color. It was found that dilute eye-color characters were accompanied by diluted ocellar color. Pink-eyed flies had ocelli about as pale as those of vermilion; however, maroon-eyed flies have ocelli only very slightly paler than the wild-type. The ocellar dilution of both pink and vermilion is thus disproportionately greater than the eye dilution, while in the case of maroon the two effects are of like degree. In the case of purple eye-color, which is darker in color than the wild-type but more translucent, the ocelli were slightly diluted. The ocelli of the very dark eye-color "sepia" seem to be about the color of the wild-type. The ocellar color of the autosomal vermilion-like eye-colors (scarlet, cinnabar, cardinal) is also white (plate 1, figs. 11 and 12).

WHITE-OCELLI IN THE SPINELESS STOCK.

The stock of the third-chromosome recessive "spineless" was found by Bridges to be pure for a white ocellar color (July 1914).

CHROMOSOME OF WHITE-OCELLI.

That the gene for the white-ocelli of the spineless stock was in the third chromosome was inferred from the observation that in experiments in which this stock of spineless had been used the white-ocelli character was not assorting independently of the spineless. No counts were made, but it was apparent that nearly all of the spineless flies had retained the white-ocelli, while a few were certainly of the wild-type.

LOCUS OF WHITE-OCELLI OF THE SPINELESS STOCK.

That the locus of white-ocelli is in the region close to the spineless locus was shown by the fact that in making up certain multiple stocks involving spineless, cross-overs were obtained that were known to have been very close to spineless (to the left), but these cross-overs did not remove white-ocelli from its association with spineless.

The character white-ocelli was largely neglected from the time of its discovery in the spineless stock until the third chromosome was fairly well mapped. One of the reasons of this neglect was the fact that the most important character of the third chromosome was pink, and the very pale ocelli of pink excluded the use of these two characters in experiments in which complete separations must be made. Furthermore, the examination of all flies during this period was carried out by the aid of a hand lens only, and the separation of white-ocelli from normal ocelli was difficult because of the minute size of the region affected. The later work has been done with a binocular microscope, with special attention to proper illumination and magnification, and under these conditions the separation is complete and entirely accurate, though still somewhat slow.

THE LINKAGE OF WHITE-OCELLI TO DICHÆTE AND HAIRLESS.

In determining the locus of spineless accurately, use was made of the dominant characters Dichæte and Hairless, which had been recently found and mapped in the third chromosome. The locus of Dichæte was near the left

end of the map of the third chromosome as then known (about 12 units to the right of sepia), while the locus of Hairless was somewhat to the right of the middle (about 37 units to the right of sepia and 20 to the left of rough). These loci were well spaced, so that a single experiment involving both Dichæte and Hairless would enable one to place a new mutant with fair accuracy no matter where its locus in the third chromosome might be. In the case of white-ocelli an even more accurate determination could be made, since there was on hand a double-recessive stock, spineless white-ocelli. Spineless white-ocelli males were crossed to females heterozygous for Dichæte and Hairless (culture 8782, September 9, 1918) giving DH 84, + 112, D 23, H 31, recombination per cent 21.6. Back-cross tests of the F_1 Dichæte Hairless females were carried out (table 28).

TABLE 28.—*P_1, Dichæte Hairless ♀ × spineless white-ocelli ♂; B. C., F_1 Dichæte Hairless ♀* $\left(\frac{D \quad H}{ss \quad wo}\right)$ *× spineless white-ocelli ♂.*

Sept. 27, 1918.	0		1		2		3		1, 2		1, 3		2, 3	
	D H	ss wo	D ss wo	H	D wo	ss H	D H wo	ss	D ss H	wo	D ss	H wo	D	ss H wo
8,793	69	79	8	16	13	10	7	7	1	1	....	1	....	1
8,841	87	38	16	9	22	7	8	3	1	1	....	....	....	....
8,842	94	48	13	5	13	7	9	7	....	....	....	1	....	....
8,843	95	93	8	10	21	12	5	7	1	....	....	....	....	....
8,845	94	84	21	11	15	15	5	10	....	1	2	4	....	....
8,848	101	82	3	15	11	17	7	8	....	....	....	2	....	....
8,849	120	104	19	14	21	19	9	11	2	1	....	....	....	....
Total	660	528	88	80	116	87	50	53	5	4	2	8	0	1

The order of three of the four loci was already known to be Dichæte, spineless, Hairless, and the results of table 28 showed that the locus of white-ocelli is to the right of that of Hairless. On the basis of the 1,682 back-cross flies, of which 114 represented recombination for Hairless and white-ocelli, the Hairless white-ocelli recombination per cent was 6.8. There is no double crossing-over within this Hairless white-ocelli section. There was only one double recombination for the spineless white-ocelli section, which is about three times as long. The value 6.8 is therefore a direct measure of the map-distance between Hairless and white-ocelli.

THE LOW COINCIDENCE OF THE HAIRLESS ROUGH REGION.

The important point in connection with the locus of white-ocelli, as thus established, is that it is situated in what had been the longest unoccupied region of the third chromosome. There had been no satisfactory mutant in the entire distance of about 20 units from ebony (1.2 units to the right of Hairless) to rough (21.6± units to the right of Hairless). The ebony rough distance was so great that in constructing a map a correction was required on account

of double crossing-over, but the amount of this double crossing-over was very problematical. It had been found for the second-chromosome that the amount of double crossing-over varied according to the region, being very high for the middle of the chromosome and very low for the ends. It was not therefore permissible to assume that the correction for the ebony rough section was of the same amount as that for some other region of the third chromosome that gives the same amount of recombination.

The presence of white-ocelli in the ebony rough region gave an opportunity to make a direct test of the amount of double crossing-over, and consequently of the amount of correction required. A spineless white-ocelli male was outcrossed to a Hairless rough female and the F_1 Hairless females were back-

TABLE 29.—*P_1, Hairless rough ♀ × spineless white-ocelli ♂; B. C., F_1 Hairless ♀ $\left(\frac{ss \quad wo}{H \quad ro}\right)$ × spineless white-ocelli rough ♂.*

Feb. 25, 1919.	0		1		2		3		1, 3		2, 3	
	ss wo	H ro	ss H ro	wo	ss ro	H wo	ss wo ro	H	ss H	wo ro	ss	H wo ro
9,432.....	95	120	16	15	8	12	22	24	2	.....	.....	.....
9,434.....	107	92	17	16	13	18	24	23	1	.....	.....	.....
9,435.....	95	103	20	14	8	6	22	31	1	1	.....	.....
9,436.....	90	89	6	18	11	8	11	11	.....	1	.....	.....
9,438.....	106	118	16	27	15	9	18	28	2	.....	.....	.....
9,453.....	89	106	17	17	4	3	20	23	2	2	.....	.....
9,455.....	99	112	12	19	13	11	27	25	2	.....	.....	.....
9,459.....	112	83	22	8	11	5	22	23	.....	1	.....	.....
9,522.....	120	120	16	26	18	4	28	15	.....	1	.....	1
9,523.....	104	121	11	22	12	8	18	34	1	2	.....	.....
9,525.....	67	62	10	13	5	7	14	18	.....	1	.....	.....
Total...	1,084	1,126	163	195	118	91	226	255	11	9	0	1

crossed singly to spineless white-ocelli rough males. The two latter stocks were made up specially for these tests. The back-cross results (table 29) gave a total of 3,179 individuals, of which 210 or 6.6 per cent were recombinations for Hairless and white-ocelli. This value agrees with the 6.8 of the previous experiment. There were 502 recombinations for white-ocelli and rough, and the percentage 15.8 is in agreement with the expectation from the usual value of 22± for Hairless rough.

There was only a single double recombination for the Hairless white-ocelli rough section, which is remarkably low in amount. This result suggests that in the third chromosome, as well as the second, the regions near the ends of the chromosome have a far lower "coincidence" than has the mid-region. The coincidence value of 3.0 $\left(\frac{3,179 \times 1 \times 100}{210 \times 503} = 3.0\right)$, is among the lowest known for regions giving recombination as great as 20.

Standard back-cross tests involving the ebony rough interval have produced 28,790 flies, of which 5,775 or 20.0 per cent were recombinations. This mean recombination per cent of 20.0 may be corrected fairly accurately by means of the following equation:

$$V = \frac{1 + \sqrt{1 - 2CR}}{C}$$

where V = the map-distance or cross-over value, C = the coincidence, and R = the recombination, all these values being used as decimal fractions rather than as percentages. The value corrected according to this formula is approximately 20.1. The amount of correction can be more directly obtained by doubling the percentage of double recombination observed. Thus, one double recombination in the total of 3,179 flies requires a correction of 0.063 per cent, or somewhat less than one-tenth of a unit. The 20.0 thus becomes 20.1, which is the same result as that obtained by the formula.

THE PERSISTENCE OF WHITE-OCELLI IN THE BLACK STOCK.

The fact that the white-ocelli of the spineless stock was linked to spineless proved that the locus of white-ocelli is in the third chromosome. The spineless white-ocelli stock furnished a double recessive suitable for back-cross tests of the position of white-ocelli. The stock of black white-ocelli that had been isolated was thereupon discarded, since the two mutants black and white-ocelli were known to be in different chromosome. However, the original stock of black still carried white-ocelli in approximately half of the individuals. No effort was made to eliminate the white-ocelli character from the black stock, because white-ocelli did not interfere with the use or classification of any second-chromosome mutant, and the viability of the white-ocelli flies seemed in no way inferior to that of the not-white-ocellars.

In December 1918, Bridges found that the third chromosome recessive "sepia" had appeared in the black stock as a result of a new mutation of sepia (see p. 88). The sepia flies were also white-ocelli. This meant that the sepia remutation had probably occurred in a chromosome that already carried the white-ocelli mutation. A census of the flies of the black stock showed that approximately half were white-ocelli. That is, the character white-ocelli had persisted in undiminished frequency from June 1912 to May 1919.

During this period the black stock had been carried on in mass-culture. Every two weeks a new culture was started by transferring, without selection, enough flies to insure breeding (from a dozen to 200). In such mass cultures overcrowding is extreme, and in spite of the great numbers of parents, not many more offspring succeed in hatching than hatch from successful pair-cultures. The competition grows keener with the age of the culture, since the number of larvæ is continually increasing from the eggs laid each day, the quantity of available food steadily diminishes, and the quality becomes progressively poorer. The mass-culture method of breeding thus exercises a strong and continuous selection against the perpetuation of the weaker or slower-hatching individuals or types.

In several instances (for example, see Whiting, 1913) mixed stocks have been started with equal numbers of different mutations, and this stock transferred without selection through several generations. Watch has been kept

and in these cases there has been a progressive change in the composition of the stock, rapid at first, until the numbers of one type were quite small, and thereafter slower but in the same direction. Recessive characters of very low viability may persist for many generations as a small proportion of the population. Their existence is maintained by the intercrossing of the heterozygotes, whereby the mutant gene escapes the adverse selection that the mutant character suffers. Certain of our mutations are so sensitive to larval overcrowding that the ratios in mass-cultures and in pair-cultures seem to belong to different systems of heredity. Thus, the character "strap" approaches 1 in 4 in pair-cultures of F_2, but may approximate 1 in 16 in sister mass-cultures.

The persistence of the white-ocelli character in undiminished proportion through 175 generations of forced competition means that the mutant is under no disadvantage. Such a mutant might easily survive in nature, and one slightly advantageous might ultimately displace the original type. It is to be noted that the character white-ocelli is one of slight extent, and in general it is probable that the mutations that do not involve great character changes are most likely to survive competition.

THE IDENTITY OF LOCUS OF WHITE-OCELLI OF BLACK AND OF SPINELESS STOCKS.

It had been assumed that the white-ocelli character that was present in the spineless stock was the same as that present in the black stock. That they were at least allelomorphic was proved by direct crosses between the two, for the F_1 flies possessed white-ocelli. No difference between these two characters has been found that would suggest that they were allelomorphs rather than identical.

An attempt has been made to trace the pedigrees of the two lines in which the white-ocelli appeared. The black stock was the original stock isolated by Morgan in November 1910, and which came from a cross between miniature and wild. The miniature in turn came from the Beaded stock. The spineless stock was derived from a single female found by Bridges in a stock with broken abdominal bands. This stock had been isolated by Morgan some months previously, but its origin is uncertain. While there was no connection traced between the two stocks, black and spineless, it is still possible that the two were related, and that a single mutation produced the white-ocelli character present in each. On the other hand, there are many proved cases of remutations of certain characters, and it is as probable that the two were of distinct mutational origin.

THE MODIFICATION OF EOSIN EYE-COLOR BY WHITE-OCELLI.

The examination of the various stocks of eye-color mutations showed that there is a strong correlation between the eye-color and the ocellar color. This direct effect of eye-color genes on ocellar color suggested that the reverse relation might also hold, namely, that the white-ocelli gene might dilute the eye-color. A careful examination of the eye-color of white-ocelli flies did not bring to light any certain effect. The white-ocelli was then crossed to vermilion, and the F_2 vermilion white-ocelli flies were not distinguishable from the simple vermilion flies. But in like F_2 cultures from the cross of

white-ocelli to eosin, a definite modification of the eosin character by the white-ocelli gene was observed (table 30). In the case of the males, the eye-color of the double form was lighter in intensity and less yellow in tone than that of the eosin brothers. In the females, the change was in the same direction, but was less marked in degree. Probably 95 per cent of the diluted males were separable from the simple eosin, while not more than 60 per cent of the females were thus separable. Eosin is known to be especially subject to specific modification (see Jour. Exp. Zool., July 1919), and the effects of the white-ocellar gene give a color intensity and pink tone and a sexual difference practically identical with effects observed in the case of the

TABLE 30.—P_1, *eosin* ♀ × *white-ocelli* ♂; F_1 + ♀ × F_1 w^e ♂ (F_2 *not-*w^e *discarded*).

Jan. 3, 1920.	w^e ♀	w^e wo ♀	w^e ♂	w^e wo ♂
11,146.....	77	13	58	24
11,147.....	50	13	59	17
11,149.....	91	11	84	22
Total.....	218	37	201	63

modifier "pinkish." The gene for pinkish was, however, in the second chromosome, and there are other differences between the two cases.

EVALUATION OF WHITE-OCELLI.

White-ocelli is strictly recessive, does not fluctuate in degree of development, can be separated from the wild-type with perfect accuracy, though not with as great speed as desirable, conflicts with the use of light eye-colors only, is of entirely normal viability, fertility, and productivity, and has a favorable position in the third chromosome.

A condensed account of white-ocelli has appeared in Biological Bulletin, April 1920, pp. 231–236.

KIDNEY (k).

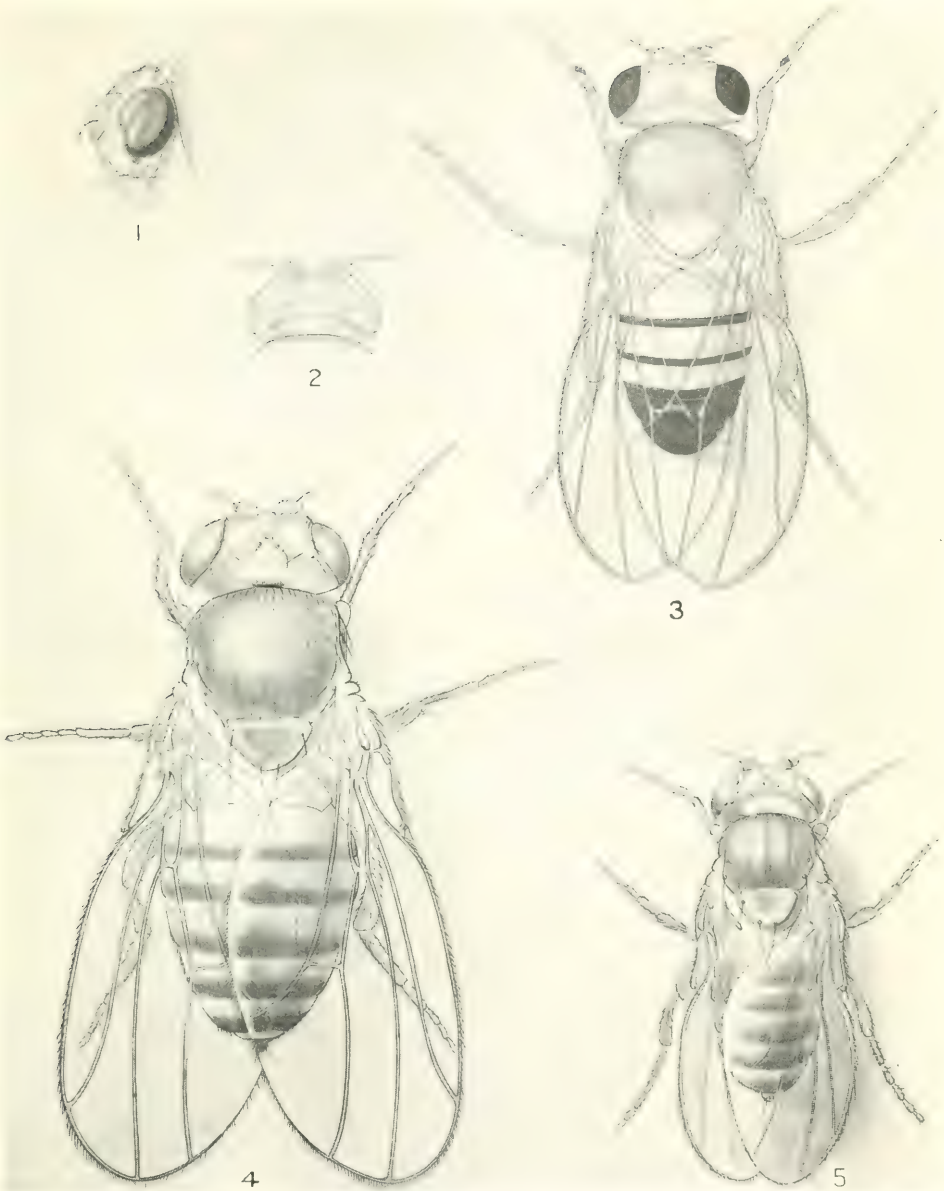
(Figure 7, page 77; Plate 3, Figure 1.)

ORIGIN OF KIDNEY.

In the first back-cross tests of males heterozygous for purple and vestigial, Bridges found a few flies whose eyes had a deep notch in the lower anterior margin (June 26, 1912; B 10. 2). These flies appeared in several cultures, and the mutation must therefore have occurred in the (vermilion) purple vestigial stock and early enough to have become quite abundant. (See Carnegie Publication No. 278, p. 176.)

CHROMOSOME OF KIDNEY.

Since the "kidney" character appeared in all the classes with respect to purple and vestigial it was concluded that kidney was probably not in the second-chromosome.



1. Kidney eye. 2. Spineless glass. 3. Spineless rough. 4 Giant. 5 Dwarf scotia.

At that time the only mutant known to be in the third-chromosome was pink, the third-chromosome having been arbitrarily defined as the chromosome that carries the gene for pink. Maroon was thought to be in the third-chromosome, but the evidence was not regarded as conclusive. (See p. 54 of this paper).

Some kidney-eyed males that did not show purple or vestigial (from B. 10) were mated to pink females. Several pairs of the F_1 wild-type flies were mated, and in F_2 the kidney character reappeared, in somewhat less than a quarter of the progeny. There was much variation in the kidney character; quite a few showed only a faint seam through the eye. In some of the F_2 cultures, purple was present with pink, and in these cases the classification was uncertain and the F_2 results rather confusing. As nearly as could be determined, however, there were no, or not more than a very small proportion of, pink kidney flies in F_2 (July 23, 1912). At that time it was not known that in the male there is no crossing-over (see Carnegie Publication No. 278, pp. 174–176), and accordingly these results were interpreted as meaning that the gene for kidney is in the third chromosome, closely linked to pink. This happens to be the fact, but we now know that no evidence on the amount of crossing-over is furnished by such F_2 results.

Sturtevant, working at Kushla, Alabama, had only a few days before this obtained a 2:1:1:0 ratio in the F_2 from pink by ebony (July 15, 1912), so that the third chromosome was thus shown to contain pink, maroon (?), ebony, and kidney.

In order to secure a stock of the double recessive pink kidney with which to conduct back-cross experiments, several matings were made between F_2 pink and F_2 kidney flies. A small proportion of such flies come from crossover gametes and give rise to flies showing both pink and kidney characters. In order to eliminate purple from the stock, the F_2 flies used in these matings were taken from a culture that had not shown purple. One of the F_3 cultures gave pink kidney flies, and these were found to be free from purple.

A pink kidney male was out-crossed to a wild female, and both male and female back-cross tests were carried out (November 4, 1912). In the male test (culture C120, table 31) no recombinations occurred. In the female test (cultures C57 and C121, table 32) both recombination classes were represented (p=47, k=37). The per cent of recombination was 14.2, figured on the basis of the kidney flies only.

Shortly after this, Sturtevant carried out back-cross tests both of the male and of the female in the case of pink ebony. He found the recombination per cent for pink ebony to be about 2. Consequently, it was thought that the locus of kidney was further from pink than was that of ebony (see Biol. Bull., 1914, p. 204). This has turned out not to be the case; the pink ebony value obtained was abnormally low because of the presence of a genetic crossover variation (CIII).

PINK KIDNEY MALE BACK-CROSS TEST—NO CROSSING-OVER IN THE MALE.

The pink kidney and pink ebony male tests just referred to were the first good evidence which showed that in the male there is no crossing-over in the

third chromosome. It was thought worth while to repeat on a larger scale the pink kidney male back-cross test. Accordingly nine further cultures of that type were raised, and in all 771 flies were obtained, none of which involved crossing-over (table 31). In culture III 14, 3, and in III 31, 2 flies were

TABLE 31.—P_1, *pink kidney* ♂ × *wild* ♀; *B. C.*, F_1 + ♂ × *p k* ♀.

Nov. 4, 1912.	p k	+	p	k	Continued.	p k	+	p	k
C 120	23	23	..	..	III 29	17	22	..	..
III 9	9	11	..	..	III 30	58	63	..	..
III 14	50	56	..	..	III 31	6	11	..	..
III 15	61	91	..	..	III 32	114	142	..	..
III 16	8	6	..	..	Total	346	425	0	0

found that seemed to be not-pink kidney recombinations. Fortunately, two of these were tested, and were proved not to be recombinations. They were wild-type flies which showed a slight seam, as wild-type flies, especially those hatched early in cultures, are now known to do occasionally. Or perhaps the kidney was due to a kidney-like mutation that was not allelomorphic to the original kidney.

Since these early experiments, much more material has been collected that fully establishes for the third chromosome the fact that there is no crossing-over in the male.

FURTHER PINK KIDNEY RECOMBINATION DATA.

From the same F_1 cultures used in starting the above male back-cross test, females were back-crossed to pink kidney males (III 1–III 27, table 32). In-

TABLE 32.—P_1, *pink kidney* ♂ × *wild* ♀; *B. C.*, F_1 + ♀ × *p k* ♂.

Nov. 4, 1912.	p k	+	p	k	Continued.	p k	+	p	k
C 57	138	195	41	33	III 17′	84	98	27	12
C 121	33	28	6	4	III 21′	117	123	30	12
III 1	122	182	37	28	III 23′	60	79	21	4
III 12	74	97	33	14	III 25′	90	107	14	22
III 19	50	93	19	7	III 27′	105	121	30	21
III 17	97	109	11	14					
III 21	104	131	18	18	17′–27′	456	528	122	71
III 23	91	111	23	6					
III 25	92	85	24	17	17–27	522	608	109	75
III 27	138	172	33	20					
Total	939	1,203	245	161					

cluding the two previous cultures (C 57 and C 121), there were 161 not-pink kidney recombinations in a total of 1,100 flies with kidney eyes. The percentage of recombination is therefore 14.6. A few F_2 cultures were also raised

(table 33), in which there were 46 kidney and 256 pink kidney flies. This result gives a recombination per cent of 15.2.

TABLE 33.—P_1, *pink kidney* ♂ × *wild* ♀; F_2, F_1 + ♀ × F_1 + ♂.

Dec. 9, 1912.	+	p	k	p k
C 145	215	25	19	102
C 184	304	30	7	72
III 2	102	12	9	31
III 4	173	13	11	51
Total	794	80	46	256

VARIATION IN CROSSING-OVER WITH AGE.

In order to obtain from each female a greater number of offspring, and hence a more representative ratio, second broods were usually raised by transferring to a fresh culture-bottle each pair at the end of the first ten-day period of egg-laying. This method had already led to the discovery that in the second-chromosome the amount of crossing-over changes with the age of the mother, so that second broods give lower recombination percentages than do first broods from the same parents (Bridges, 1915, J. E. Z.).

From 5 of the pink kidney back-cross pairs (17–27, table 32) second broods were raised. The results seem to show that there was a slight rise in the amount of recombination given in the second broods. But when the recombination per cents are calculated on the basis of the kidney flies alone, which is now known to be a more accurate method, the descrepancy is only 1 unit, which is by no means significant. Further work on the question is being carried out by Bridges and by Dr. H. H. Plough.

LINKAGE OF KIDNEY WITH SOOTY AND SPINELESS.

During the spring of 1914, Dr. H. J. Muller did much work on the localization of third-chromosome genes. He made up several multiple stocks, in-

TABLE 34.—P_1, *peach kidney sooty* ♂ × *wild* ♀; *B. C.*, F_1 + ♀ × $p^p\ k\ e^s$ ♂ (*July 7, 1914*).

$p^p\ k\ e^s$	+	p^p	$k\ e^s$	$p^p\ k$	e^s	$p^p\ e^s$	k
79	76	11	7	8	15	..	..
40	39	8	6	3	3	..	..
20	40	6	7	3	4	..	..
46	74	12	11	9	15	2	..
109	151	20	14	14	20	1	..
294	380	57	45	37	57	3	0

cluding "peple" (peach spineless kidney sooty rough) and "seple" (sepia spineless kidney sooty rough). In making up the "peple" stock he observed the recombination for peach, kidney, and sooty in several cultures (table 34).

The peach kidney recombination per cent was 12.0 and the kidney sooty value 9.8.

DEALING WITH AN OVERLAPPING CHARACTER.

Recently, Bridges carried out two other experiments in which the relation of kidney to spineless and to ebony was carefully tested. In the first of these,

TABLE 35.—P_1, *pink kidney* ♂ × *spineless* ♀; *B. C.*, F_1 + ♀ × "*peple*" ♂.

Oct. 29, 1918.	p k	k	ss k	p ss k	ss	p ss	p	+
8,917.....	83	12	4	1	102	16	20	3
8,918.....	72	5	6	..	91	11	15	4
8,919.....	70	10	11	1	104	19	16	1
8,920.....	84	14	9	1	133	15	24	4
Total.....	309	41	30	3	430	61	75	12

a pink kidney male was mated to a spineless female, and F_1 wild-type females were back-crossed singly with "peple" males (table 35). In the second, a spineless kidney sooty male was crossed to a wild female, and F_1 females were back-crossed to "peple" males (table 36).

TABLE 36.—P_1, *spineless kidney sooty* ♂ × *wild* ♀; *B. C.*, F_1 + ♀ × "*peple*" ♂.

Dec. 4, 1918.	ss k e^s	k e^s	ss k	k	+	ss	e^s	ss e^s
9,099.....	53	6	2	..	123	11	11	62
9,100.....	62	5	4	..	149	10	19	69
9,101.....	70	6	..	..	161	16	14	87
9,102.....	68	3	11	..	173	12	15	78
9,103.....	82	7	9	..	196	11	16	82
9,104.....	77	13	5	..	138	13	18	34
9,105.....	49	4	2	..	110	8	9	48
9,106.....	63	5	2	..	137	17	17	66
9,113.....	70	4	5	..	148	4	8	60
9,114.....	83	2	5	..	131	7	11	66
9,115.....	57	6	3	..	136	9	17	66
Total...	734	61	48	..	1,602	118	155	718

Since the kidney character overlaps the wild-type character, it is not possible to be sure that the kidney versus not-kidney separation is complete. Accordingly, in making the classifications of tables 35 and 36, the first separation made was that of kidney versus non-kidney. This separation was carried out with a complete disregard to the other characters present. Furthermore, only those flies about which there was no doubt were included in the kidney class. The separation was carefully checked up by reexaminations. That is, all flies classified as kidneys were kidney, though the flies classified as not-kidney

included a varying proportion of flies that were genetically kidney. Among the kidney flies, some are pink kidney (original combinations), and some are spineless kidney (recombinations), etc.

The recombination percentages in the first experiment are: p-ss 12.2, ss-k 8.6, and p-k 18.5, and in the second, ss-k 7.2, k-e 5.7, and ss-e 11.1. The p-ss and ss-e values are calculated from all the flies of the respective experiments, since there is no error in the classification of these characters. The values involving kidney are calculated from the kidney flies only.

LOCUS OF KIDNEY.

On the basis of all the recombination data involving kidney, the locus of kidney is about 7 units to the left of ebony and 5 units to the right of spineless, or 64 units to the right of roughoid.

DESCRIPTION OF THE KIDNEY CHARACTER.

The first kidney flies found (B 10) had eyes with a strong indentation in the anterior ventral part of the eye (figs. 7*a*, 7*b*, and 7*c*; plate 3, fig. 1).

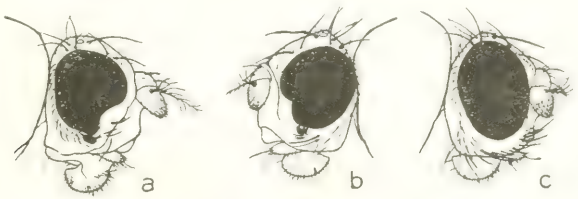


FIG. 7.—Kidney eye. (a, b) Typical kidney eyes; note tuft of hairs below eye. (c) Kidney character reduced to a slight seam in the middle of the eye.

Only about three-fourths of the area of the eye remained. It was soon noticed that the two eyes were often quite different in the degree of the indentation, and that there was great variation from fly to fly. In reduction, the eye varied to only about a quarter of the normal area, the postero-dorsal region being relatively unaffected.

The small bristles that are normally present on the cheek below the eye often become congregated into a tuft below the indentation (figs. 7*a*, 7*b*). This tuft is less often present in flies in which the eye is much reduced than in flies of medium or of rather slight development of the indentation.

The lower part of the eye bordering the indentation, or in fact all of the eye except the postero-dorsal portion, may be sunken to the extent that the eye is in two distinct levels.

The variation in the opposite direction overlaps the normal. The slightest trace of the kidney character is a tiny pit or seam in the middle of the eye (fig. 7*c*, above). This seam may run forward and join with a slight indentation at the anterior margin. On opposite sides of this seam there is usually a lack of correspondence in the rows of ommatidia.

There is a curious relation between the degree of development of this character and the age of the culture. Among the first flies that hatch in the culture all the kidney flies are easy to distinguish, and the separation is complete. In from 4 to 6 days the grade of the character has become lower, so that some of the kidneys remain undetected. In the later and last counts still fewer flies show extreme indentations, and the proportion of flies in which the kidney can not be detected may reach to 50 or more per cent. That the relative abundance of kidney flies in the early counts is not to be explained by assuming that kidney flies undergo development at a faster rate than normal and consequently hatch earlier, is shown by the fact that in these early counts the number of kidneys is not in excess of the number of not-kidneys, but is in the expected equality with it. The deficit of kidneys in the later counts is not due to poorer viability of kidney. Since there is no distortion to the ratios among the other linked characters present, such as pink and spineless. The linkage relations and direct tests have shown that flies that are genetically kidney may fail to show the kidney character.

Morgan had observed a similar variation in the case of abnormal abdomen (J. E. Z., 1915), where all the flies "change over," in the course of the counts from Abnormal to wild type. By adding fresh food, the changing over can be prevented; the sensitive period is apparently during the early pupa stage, as judged from the amount of time from the adding of fresh food to a change in the ratio.

EVALUATION OF KIDNEY.

Kidney was one of the mutants that were found relatively early, and it, therefore, is connected with some of the pioneer work on autosomal linkage and in the mapping of the third-chromosome; but its importance has no longer been maintained, because, in the first place, the character fluctuates greatly in degree of development, and overlaps the wild-type increasingly as the cultures become older, thus decreasing its value very greatly in spite of the excellent viability, fertility, and other good characteristics of the race. In the second place, the locus of kidney is about halfway between those of two mutants, spineless and ebony, that are excellent in every respect, and that are so close together (about 12 units) that double crossing-over between them is negligible. More recently there have been found in this same section of the chromosome an excellent dominant, Delta, and two recessives, glass and stripe, all of which are more useful than kidney.

Owing to these drawbacks to the usefulness of kidney, it does not seem worth while to keep the mutant any longer; but since the character is in the seple and peple multiples, and in other stocks that have been derived from them, it will be difficult and laborious to rid our stocks of its presence.

BAND (bn).

(Figure 8.)

ORIGIN OF BAND.

In selecting for darker types of the trident-pattern mutant "with" (see p. 31), Morgan found (about July 1912) a few individuals in which the prongs of the trident were much heavier than normal, and in which there was a heavy dark band across the anterior part of the thorax. These individuals were bred together and a pure-breeding stock was produced.

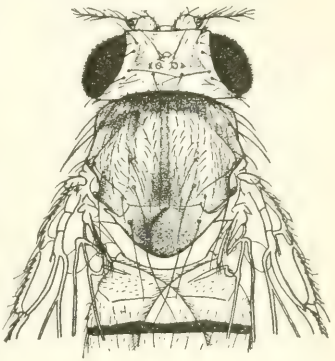


FIG. 8.—Band thorax-pattern.

INHERITANCE OF BAND.

Crosses between "band" male and "with" female were carried out on an extensive scale, with the result that the F_1 flies were usually all "with," but in a few cases in which the "with" had been heterozygous for band, half the flies were band and half were "with." The same results were obtained in crosses of band female to "with" male. Evidently the band character is recessive and not sex-linked. The band character, as found, was thus a double-mutant type, produced by the semidominant gene "with," and the recessive gene band.

There was considerable variation in the intensity of the band character, and much of this was genetic, since selection soon established a "faint-band" and "broad-band" stock.

CHROMOSOME OF BAND.

Band was crossed by Bridges to maroon, and in F_2 there were no maroon band (or maroon "with") flies. A similar cross of band to pink produced no pink band (or pink "with") F_2 individuals (table 37). These crosses proved that band and "with" were third chromosomal.

TABLE 37.—*P_1, pink ♂ × band ♀; F_2, F_1 + ♀ × F_1 + ♂.*

Jan. 9, 1913.	+	p	bn	p bn
III 5	104	38	48	.....
III 6	67	32	18	.....
III 6′	319	114	126	.....
III 7	304	164	107	.....
III 7′	111	56	57	.....
Total	905	404	356	.....

LOCUS OF BAND.

From the F_2 flies of the above crosses matings were made that gave the double-recessive stocks maroon band and pink band.

A maroon band female back-cross test was carried out, and this gave 20.8 per cent of recombination for maroon and band (table 17).

The pink band stock was crossed by Muller to the pink kidney stock and a pink kidney band stock occurred. A back-cross was made (table 38) that showed that the locus of band is to the right of kidney by about 8 units, or very close to the locus of ebony. This position agreed with the previously found maroon band recombination per cent of about 21.

TABLE 38.—*Pink kidney band, ♀ B. C.* (*By Dr. H. J. Muller, April, 1915*).

0	1	2	1, 2	Total.
192	25	18	2	237

An accurate determination of the locus of band was not made until after the discovery of the dominant character Hairless (see p. 161), whose locus is in the third chromosome close to that of ebony and the supposed locus of band. A pink band female was crossed to a male which carried the genes for Dichæte and for Hairless in the same third-chromosome homolog. F_1 Dichæte Hairless females were then mated to pink band males (table 39). The crossing-over

TABLE 39.—P_1. *Dichæte Hairless ♂ × pink band ♀; B. C.,* F_1 $\frac{D}{p}\ \frac{H}{bn}$ ♀ × *p bn ♂.*

Sept. 30, 1918.	D H	p bn	D p bn	H	D bn	p H	D H bn	p	D p H	bn	H bn	D
8,799.....	164	113	6	11	26	54	4	4	..	..	..	..
8,800.....	97	106	4	4	20	27	3	4	..	3	..	1
8,801.....	110	141	7	10	48	40	3	3	2	1	1	1
8,802.....	117	148	5	10	39	36	5	5	1	2	1	..
8,803.....	107	106	11	9	28	24	2	2	..	3	..	..
8,804.....	95	124	10	8	24	23	4	2	..	4	..	1
Total....	690	738	43	52	185	204	21	20	3	13	2	3

relations showed that the position of band is to the right of Hairless by about 2.3 units.

A back-cross of pink band by the dominant mutant Delta (see page 199) gave a position for band in agreement with the above (table 142). The indicated locus of band was thus only slightly to the right of that of ebony, and it was considered possible that band might be an allelomorph of ebony. Crosses between band and the various allelomorphs of the ebony locus were carried out, and these gave in F_1 only wild-type flies, showing that the loci are distinct.

DESCRIPTION OF BAND.

The band character present in the pink band stock is slightly different from the band as originally found, which difference is to be attributed to the modi-

fication by "with" in the original stock. In the "with" band stock the prongs of the trident were well developed and the space between the side prongs was, in the more extreme individuals, completely filled in, so that there was present a broad black band extending from the scutellum to the anterior end of the thorax.

In the band of the pink band stock the mid-prong is broad and dark, but the side prongs are relatively weak. The most conspicuous feature, and the one most useful in classification, is the heavy dark band across the anterior end of the thorax. Usually this transverse band is confined to the space between the side prongs that reach forward to the anterior margin of the thorax, but often it extends beyond the trident prongs down on the sides of the thorax. In the usual type of band the pattern resembles two tridents, one directed anteriorly, the other posteriorly, that are fused point to point.

The pattern described above appears to be due to pigment deposited in the hypodermis beneath the chitinous cuticle. The cuticle itself appears rather thin and weak. The transverse suture on the thorax is much deeper than normal and is underlaid with pigment. There is also often a median longitudinal crease or slight depression at the anterior end of the mid-prong. The thorax itself is slightly flattened, and there is a vacuolated appearance to the muscular tissues within.

The changes in the interior of the thorax and also in the pattern are very similar to the characteristic of the second-chromosome dominant character streak (see p. 223, Carnegie Pub. No. 278). A histological examination has been made of the interior of the thorax of these two forms, and it is apparent that the musculature is deficient, the space normally occupied by muscles being partly filled with bubbles and with blood-sinuses.

There is a slight disarrangement of the bristles and hairs of the thorax as follows: The distance between the rows of the microchæte is increased in the middle of the thorax, so that the rows diverge from the scutellum to this point and converge from this point to the anterior margin of the thorax. The individual hairs between the dorso-central bristles point medially instead of posteriorly, and this is somewhat true of the dorso-central bristles themselves.

There is considerable fluctuation in all these characteristics, but the mutant can nevertheless be distinguished from the wild-type with accuracy and with fair ease.

PINK BAND × HAIRLESS; SOOTY × HAIRLESS.

Incidental to making up a Hairless band stock for a closer determination of the locus of band, two Hairless by pink band back-cross cultures were raised (table 40). There was 2.8 per cent of recombination for Hairless and band.

TABLE 40.—P_1, *Hairless × pink band; B. C., F_1 H ♀ × p bn ♂.*

Oct. 27, 1920.	p bn	H	p H	bn	p	H bn
12,068	46	58	15	8	2	
12,069	42	46	13	13	3	2
Total	88	104	28	21	5	2

Likewise, two cultures of Hairless × sooty back-cross were raised (table 41), which gave only 0.5 as the percentage of recombination. If these data are comparable, the locus of band is about 2.4 units to the right of that of sooty.

TABLE 41.—P_1, *Hairless band×sooty; B. C., F_1 H ♀×e^s ♂.*

Nov. 20, 1920.	H	e^s	H e^s	+
12,145	159	198	3	2
12,180	193	181	1	2
Total	352	379	4	4

Parallel back-cross tests of females carrying Hairless and band in one chromosome and sooty in the other failed to produce any offspring in the tests with sooty males, and produced only a few in the tests with pink band males (table 42).

TABLE 42.—P_1, *Hairless band×sooty; B. C., F_1 H ♀×p bn ♂.*

Dec. 24, 1920.	H bn	+	H	bn	Cont.	H bn	+	H	bn
12,270	5	12	..	..	12,287	15	19	1	..
12,271	6	4	..	..	12,288	14	16	..	1
12,286	23	31	1	..	Total	63	82	2	1

The locus of band on the basis of all the data is at about 2.5 units to the right of Hairless or 1.3 units to the right of ebony.

EVALUATION OF BAND.

The viability of band is inferior to that of the wild-type, but only slightly so. The other characteristics of the mutant are good. The locus of band is so close to that of ebony and Hairless that it is not likely that band will be used, except in very special studies such as tests of the extent of a "deficiency." (See Bridges, 1917 and 1919.)

PEACH (p^p).

(Plate 1, Figure 6.)

ORIGIN OF PEACH.

The newly-discovered mutant "dachs" had been crossed to the second-chromosome recessive black, and the cross had given a 2 : 1 : 1 : 0 ratio in F_2 (see p. 217, Carnegie Pub. No. 278). Hence the locus of dachs was known to be in the second chromosome. The double recessive dachs black was necessary in order to carry out tests as to the relative position of the dachs locus. F_2 dachs and black flies accordingly were bred together. In F_3 a few dachs

black flies were secured. But in one of the F_3 cultures Bridges found a few flies with a yellowish pink or "peach" eye-color (January 24, 1913). Some of the peach flies were bred together, and they produced a pure-breeding stock of peach.

TABLE 43.—P_1, *peach* ♂ × *wild* ♀; F_2, F_1 + ♀ × F_1 + ♂.

March 1, 1913.	+ ♀	+ ♂	p^p ♀	p^p ♂
M 18.....	162	151	51	49
M 19.....	324	320	102	105
M 20.....	322	288	100	88
M 21.....	239	216	72	63
Total.....	1,047	975	325	305

A peach male was then out-crossed to a wild female, and in F_2 the peach reappeared in about one-fourth of the males and one-fourth of the females (table 43). Therefore, peach is not sex-linked. It is completely recessive in the wild-type heterozygotes (F_1 and F_2).

CHROMOSOME OF PEACH.

In order to determine to which linkage group peach belongs, peach was mated to black (second-chromosome) and to ebony (third-chromosome). The

TABLE 44.—P_1, *peach* ♀ × *black* ♂; F_2, F_1 + ♀ × F_1 + ♂.

July 4, 1913.	+	b	p^p	b p^p
D 1.....	99	35	49	18
D 2.....	159	61	43	15
Total.....	258	96	92	33

F_2 from peach by black gave a typical 9 : 3 : 3 : 1 ratio (table 44), showing that there was free assortment between black and peach, hence the peach gene is not in the second-chromosome. The F_2 from peach by ebony gave a 2 : 1 : 1 : 0 ratio (table 45), showing that the locus of peach is in the third-chromosome.

TABLE 45.—P_1, *peach* ♀ × *ebony* ♂; F_2, F_1 + ♀ × F_1 + ♂.

July 4, 1913.	+	p^p	e	p^p e
D 3.....	309	167	139	0
D 4.....	202	80	90	0
Total.....	511	247	229	0

ALLELOMORPHISM OF PEACH AND PINK

The results of the above two crosses were not unexpected, for it had already been observed that in the original culture the peach character showed no noticeable linkage to the second-chromosome characters black and dachs. On the assumption that peach would be found in the third chromosome, it became of interest to determine whether it was allelomorphic to pink, which it resembled somewhat. Accordingly, peach males were crossed to pink females. In F_1 the flies were not wild-type, but were intermediates between pink and peach, resembling peach rather than pink. Two F_2 cultures were raised (table 46), in which a fairly accurate separation of pure pink from all other flies could be made. Pink occurred in about one-quarter of the individuals only. A few pure peach flies were identified doubtfully. The F_2 ratio was found to be roughly 3 peach-like to 1 pink-like.

Since peach, so far as traceable, was independent of pink in origin, these F_1 and F_2 results showed that peach is an allelomorph of pink. In subsequent work with peach there has been nothing to indicate that peach might be pink modified by the action of a separate but linked gene. The pink-peach allelomorphism was the first case of autosomal multiple allelomorphs found in *Drosophila*. The sex-linked systems white-eosin-cherry, and yellow-spot had been found previously. Two other allelomorphs of pink (pink[3] and pink[4]) were found after peach was found.

TABLE 46.—P_1, *peach* ♂ × *pink* ♀; F_2, F_1 *peach-like* ♂ *and* ♀.

	Peach-like.	Pink.
D 9	191	91
D 10	245	86
Total	436	177

DESCRIPTION OF PEACH.

The eye-color of the flies of the peach stock is a translucent yellowish pink, lighter in tone than that of pink, and differing from pink in the direction of "orange" (the double recessive vermilion pink). The "fleck" of peach eyes is yellow but inconspicuous. Peach eye-color resembles that of eosin females very closely, but is slightly yellower and slightly lighter in tone. Peach fluctuates more in intensity than does pink, and overlaps the pink range. There is doubt in the identification in about a quarter of the flies if equal numbers of pink and peach flies of like ages are mixed. The yellow of the peach eye is especially pronounced in freshly hatched flies. As the flies become older the color becomes progressively darker and more nearly a pure deep pink or dark dull ruby. Old pink flies become a very dark sepia or purplish red. The separation of peach from wild-type is complete and more rapid and easy than the like pink-red separation. The peach gene has no other observed effect than the change in eye-color.

LOCUS OF PEACH.

Peach was incorporated with other third-chromosome recessives into the multiple recessive "peple." The recombinations percentages given by peach do not seem to be different from those given by pink. All the data obtained by using peach have been combined with the pink data in calculating the locus of pink.

EVALUATION OF PEACH.

In viability, fertility, and productivity peach is practically normal. Because the speed and ease of separating peach from wild-type is slightly better than in the like separation of pink from red, peach has been used more than pink in experiments. But pink is perhaps to be preferred on the whole. The fluctuations in the pink character are less than in peach. Also, pink is a less extreme departure from the wild-type color than is peach, and would therefore tend to interfere less with the use of other eye-colors. And it is generally true that the more extreme the character the more pronounced are the accompanying inviability effects. Peach itself has a yellowish tone, not unlike, though less extreme than the "orange" of the important double- recessive forms scarlet, peach, and peach cardinal. The substitution of pink for peach would probably make these separations easier. The separation of pink from wild-type is complete and perfectly accurate, and the speed of separation increases with familiarity.

TRUNCATE–INTENSIFIER–III.

In their experiments upon the inheritance of the character Truncate, Altenburg and Muller found (about April 1913) in the third chromosome a gene that by itself produced no detectable somatic effects, but which was an intensifier of the Truncate character.

The "chief gene" for Truncate is in the second chromosome (locus about 9 to the right of Star, rather then 28, as reported by Bridges and Morgan, or 12 as reported by Altenburg and Muller), and is a dominant, lethal when homozygous. Certain Truncate stocks bred practically true, because of the presence of a "balancing lethal" in the other member of the second-chromosome pair. The Truncate character produced by Tii is not very extreme, and is detectable only in a very small proportion of the flies. But in chromosome I, probably in II, and certainly in III, there were genes that intensified the character. These modifiers have arisen at different times, and the long selection carried out by Morgan and later by Altenburg and Muller had accumulated them and had produced a Truncate character that was an extreme departure from the wild-type.

It is probable that there were several modifiers present in the third chromosome, since cross-overs in various regions gave sections that seemed to contain an intensifier. These third-chromosome intensifiers, taken together, produced some effect in heterozygous forms, but much more in homozygous forms. Homozygous Tiii was, therefore, not lethal, but it was found to be highly infertile. A full account of this case has been published by Altenburg and Muller in Genetics, January 1920, pages 1 to 59.

SEPIA (se).

(Plate 1, Figure 2.)

ORIGIN AND INHERITANCE OF SEPIA.

In making up fresh cultures of the stocks, Miss E. M. Wallace found a few flies that had an unknown very dark eye-color (May 10, 1913). These "sepia" flies were given to Bridges, who carried out the tests described below. A sepia male was crossed to wild females. The sepia character was completely recessive. From the F_1 wild-type flies four F_2 cultures were raised (table 47),

TABLE 47.—P_1, *sepia* ♂ × *wild* ♀; F_2, F_1 + ♀ × F_1 + ♂.

June 2, 1913.	+ ♀	+ ♂	se ♀	se ♂
M 30	128	132	22	35
M 34	226	163	77	59
M 35	158	126	49	32
M 44	139	83	59	48
Total	651	504	207	174

in which the sepia reappeared as about a quarter of both males and females. The presence of the sepia granddaughters showed that the sepia character is not sex-linked. A pure-breeding stock of sepia was started from the F_2 sepias.

DESCRIPTION OF SEPIA.

Sepia is by far the darkest eye-color thus far found in *Drosophila*, especially when in old flies. The eye-color in old sepia flies is purplish black. When first hatched, the sepia flies have a deep translucent pink eye-color, very similar to that of the mutant pink. This color deepens to "maroon" and to "garnet." The typical color of flies about a day old is dark, brownish-red, and translucent, like a coffee infusion. There is no "fleck" present in sepia eyes, except in very old flies in which a dark metallic fleck appears. Aside from the age change, there is very little fluctuation in the sepia character. The separability of sepia from wild-type is complete and exceptionally easy.

CHROMOSOME OF SEPIA.

That the locus of sepia is in the third-chromosome was shown by the 2 : 1 : 1 : 0 ratio obtained in the F_2 cultures from a cross of sepia to the third-chromosome recessive ebony (table 48).

TABLE 48.—P_1, *sepia* ♀ × *ebony* ♂; F_2, F_1 + ♀ × F_1 + ♂.

Aug. 10, 1913.	+	se	e	se e
M 40	106	40	54	0
M 41	88	54	46	0
Total	194	94	100	0

LINKAGE OF SEPIA TO PEACH AND EBONY.

The efficiency of the multiple back-cross in furnishing strictly comparable data, and in providing (in the smallness of the double recombination classes) a sure index of the order of the genes had become appreciated. For this reason it was decided to carry out at once a three-point back-cross with the three genes sepia, peach, and ebony. Peach was chosen rather than pink because it was thought that the double recessive sepia peach would be easier

TABLE 49.—*P_1, peach ebony ♂ × wild ♀; B. C., F_1 + ♀ × p^p e ♂.*

Jan. 27, 1914.	p^p e	+	p^p	e
III 49.....	156	128	3	5

to separate from sepia than would sepia pink. A peach ebony stock was made up. This gave the usual value for recombination for the pink and the ebony locus (table 49). Likewise a sepia peach stock was made up in order to find out whether the difference between the double form and sepia was workable. These two stocks were crossed, and in F_2 a few sepia peach ebony flies were obtained. Three back-cross tests were carried out (see totals in table 50). These seemed to show that the locus of sepia is to the left of that of peach and ebony by a long interval. However, more difficulty than expected was found in the separation of sepia peach from sepia, and the experiment was

TABLE 50.—*P_1, sepia peach ebony ♂ × wild ♀; B. C., F_1 + ♀ × se p^p e ♂.*

Jan. 27, 1914.	se p^p e	+	se	p^p e	se p^p	e	se e	p^p
III 53+.....	135	166	54	59	1	1	..	1

discontinued. The location was not regarded as well established. It is now known that the results of tables 49 and 50 are influenced by the presence of CHH. When no cross-over variation is present pink ebony gives a recombination per cent of about 21.4. This stock was not used, for Muller soon showed (by means of the relation of sepia to the mutant, spineless, which was found shortly after sepia) that sepia is to the left of the pink locus. He incorporated sepia into a multiple stock (seple) with spineless, kidney, sooty, and rough.

LOCUS OF SEPIA.

Sepia has been used very extensively in linkage experiments; only four or five other third-chromosome loci have an equal bulk of data. All the standard data bearing on the crossing-over relations of sepia are summarized in table 2. On the basis of these data, sepia is 14.4 units to the left of Dichæte, or 26.0 units to the right of roughoid.

REAPPEARANCE OF SEPIA.

In locating the gene for the sex-linked mutant scute by means of a cross of forked male to Bar female, Bridges noticed that about a quarter of the flies of one culture (No. 4416, April 28, 1916) had "sepia"-colored eyes. This character was evidently a non-sex-linked recessive, and following the recently established procedure for determining the chromosome of a new mutant, a "sepia" male was crossed to the double dominant Star Dichæte. Also, a stock of "sepia" was made by mating together "sepia" males and females. An F_1 Star Dichæte male was back-crossed to a "sepia" female from the new stock, and at the same time a female back-cross was started. The male back-cross showed that "sepia" is in the third-chromosome. The counts of the male back-cross were immediately discontinued, but in the case of the female back-cross they were continued, disregarding the second-chromosome character Star (table 51).

TABLE 51.—P_1, *"sepia"* ♂×[*S*] *D* ♀; *B. C.*, F_1 [*S*] *D* ♀×*"sepia"* ♂.

June 19, 1916.	se	D	se D	+
4,809.....	166	163	24	19

The amount of recombination for "sepia" and Dichæte was found to be 11.5, which was the same as the value known for standard sepia and Dichæte. The character "sepia" was compared with sepia, and, as far as could be determined, was identical. Crosses were then made between "sepia" and sepia, and all the F_1 flies were sepia-like. The character "sepia" was thus proved to be a mutant in the sepia locus, and either identical with sepia or so closely similar that special methods would have to be employed to show a difference. This was not thought worth while, and the "sepia" stock was discarded.

That "sepia" had an independent origin from sepia is fairly certain. Sepia had never been crossed to any of the stocks involved in the experiment, nor had it been present as an impurity, for the Bar stock had been used constantly, with no indication of any other character, while the scute stock had been closely followed for several generations.

SECOND REAPPEARANCE OF SEPIA.

A new second-chromosome dominant, "Lobe" eye, had been found, and a back-cross of Lobe with Star black was being carried out to find the locus of Lobe within the second-chromosome, when Bridges observed a few flies with sepia-like eyes in two of the cultures. These flies appeared at random in the various classes, and the gene was therefore probably not second-chromosomal. A stock of this sepia was made, and the mutant was also crossed to the double dominant Dichæte Hairless on the assumption that the gene was in the third-chromosome. Several female back-cross tests were carried out (table 52). These gave linkage relations for the new sepia that were comparable with the expectations for sepia itself (viz., sepia Dichæte=14.0). The mutant was

crossed to sepia, and the F_1 flies were sepia. A careful comparison of the two characters showed no difference.

In the present case there is no question but that the sepia originated as a new mutation. The pedigrees of Lobe and of the Star black stocks were traced, and the stocks from which they came were examined. It was found that sepia was present in the black stock. This black stock had been maintained by simple selection for several years, and still contained the third-chromosome mutant white-ocelli (see p. 70). The sepia-like flies were ex-

TABLE 52.—P_1, *sepia* ♀ × *Dichæte Hairless* ♂; *B. C.*, F_1 *D H* ♀ × *se* ♂.

Dec. 26, 1918.	se	D H	se D H	+	se H	D	se D	H
9,219.....	73	56	14	14	16	12	..	..
9,224.....	56	57	15	13	16	26	1	..
9,270.....	97	105	13	9	32	33	5	8
9,271.....	111	116	12	18	60	41	10	7
9,272.....	45	77	13	4	12	19	3	1
9,273.....	78	63	8	6	21	21	11	5
9,275.....	103	110	22	18	35	24	2	8
9,276.....	66	68	11	15	30	28	7	5
Total.....	629	652	108	97	222	204	39	34

amined and seen to have white-ocelli. It is certain, therefore, that the mutation occurred in the black stock and probably in the flies carrying the white-ocelli gene.

EVALUATION OF SEPIA.

Sepia was for a long time the locus farthest to the left in the third-chromosome, and this very advantagous position, together with the excellent characteristics of the mutant (complete and easy separability, normal viability, fertility, and productivity) placed it among the most valuable third-chromosome mutants. Roughoid has since been discovered to the left of sepia, but the interval between roughoid and sepia (26.0 units) is so great that sepia has even an added value in bridging the gap between roughoid and the main body of genes.

Recently the very excellent mutant hairy has been found with a locus only half a unit to the right of sepia. Since hairy can be used without confusion with all other mutants, while sepia can not be used successfully with any other eye-color, it seems probable that hairy will be more useful than sepia in experiments in which this region of the chromosome is involved. In calculating locations, the data from sepia and from hairy sources can be combined.

CIII.

The first genetic cross-over modifier to be detected in *Drosophila*, and the one most used subsequently, has been called CIII. The letter C designates a cross-over modifier, and the capitalization means that it is a dominant. The Roman III designates its locus as in the third-chromosome.

CIII IN EBONY.

In making pink ebony back-crosses (see p. 52), Sturtevant found that some cultures gave 20 or more per cent of recombinations, while others gave only 1 or 2 per cent (Sturtevant, 1913). There was considerable variation, and it was some time before it was recognized that the "high" and the "low" cultures were different. Sturtevant found that sooty, an allelomorph of ebony, gave only high values (20±) with pink.

CIII IN SPREAD.

Muller, in attempting to incorporate spread into a multiple stock of third-chromosome mutants, found that flies heterozygous for spread gave the same sort of abnormal linkage relations as those heterozygous for ebony (Muller, 1916). The mutant spread had been found by Dexter in crosses of vestigial (Dexter, 1915), and vestigial had appeared in the same stock as had ebony, viz., the Truncate. The presumption was that the similar abnormal behavior of ebony and of spread was due to the same cause—the presence of an inherited dominant modifier of crossing-over.

CIII OPPOSITE BEADED.

In analyzing the constitution of a pure-breeding stock of Beaded, Muller (1919) found that whenever the not-Beaded third-chromosome was present as one of the third-chromosomes there was almost no crossing-over throughout the region from pink to rough. There was crossing-over in the region to the left of pink, and this crossing-over was normal in frequency, or even higher.

Muller called this crossing-over suppressor CIII, for the reasons given in the first paragraph of this section. The Beaded, the ebony, and the spread stock had all been derived from the same parental stock, namely, Truncate, and it therefore seemed probable that the three cross-over suppressors were the same. The fact that the pink sooty recombination per cent was reduced to about 1.5 in the presence of heterozygous CIII was in agreement with the "low" pink ebony values that had been found by Sturtevant.

Muller found, when pink ebony flies carrying the suppressor were mated to flies carrying CIII from Beaded or from spread stocks, that crossing-over in the female carrying both suppressors was at least as free as it was in the absence of either. This fact proved a special relation between the suppressors, and was taken as confirmation of the identity of the suppressors, the effect in homozygous condition being different from that in heterozygous.

CIII IN EOSIN.

In working with a "disproportionate dilution" of eosin, viz, cream–III, Bridges (1919) combined cream–III with ebony. The results obtained with this stock were in the main the low values expected from the known presence of CIII with the ebony. But some of the cultures showed high values, which might mean the unexpected absence of CIII from the cream–III ebony chromosome or the unexpected presence of CIII in the other third-chromosome. The high values in the first case would be simply "standard," but in the second case they would correspond to those for homozygous CIII. A repetition of the experiment gave the same two types of results, and the subsequent breeding

tests agreed with the supposition that the unexpected high values were due to homozygous Cili. and that the eosin stock was "impure" for Cili. Sturtevant tested flies from the eosin stock and showed that they actually were heterozygous for a cross-over suppressor indistinguishable from Cili. The connection of eosin to any other stock containing Cili was, so far as known, at least very remote. It is possible that the Cili of eosin stock represents an independent mutation.

CIII IN MAROON DWARF.

In working with the maroon dwarf stock, Bridges found that there was present a cross-over suppressor that strongly affected the region to the right of pink, and that gave free crossing-over when mated with the known Cili. That is, the cross-over suppressor in the maroon dwarf stock was allelomorphic to, if not identical with, Cili. There is no known connection between the maroon dwarf stock and the other stocks containing Cili, and, in this case also, the Cili may have arisen through an independent mutation.

CROSSING-OVER IN HETEROZYGOUS CIII.

In table 53 is summarized the different sets of data bearing upon the frequency of crossing-over in heterozygous Cili. The most characteristic recombination per cents are: sse = 0.2, ero = 0.0. From this it is seen that the greatest suppression is in the region around ebony. Between this region and rough,

TABLE 53.—*Summary of recombination data, heterozygous* Cili.

Loci (alphabetical).	Primary data.		Secondary data.	
	Per cent.	Numbers.	Per cent.	Numbers.
cr-III–D	3.9	975		
cr-III–e	14.4	2,880	8.7	975
D–dw			6.1	940
D–e	6.3	1,405		
D–H			6.1	940
D–ma	5.9	940		
D–ro			8.1	430
D–ss	7.9	430		
dw–H	0.0	940		
e–ro	0.0	1,441		
k–e	0.2	674		
k–ro	0.1	1,211		
ma–dw	0.2	940		
ma–H			0.2	940
p–e	0.9	1,901		
p–k	1.5	868		
se–D	15.3	430		
se–e			25.6	1,015
se–p	27.4	417		
se–ro			24.2	598
se–ss	22.2	1,244		
ss–e			0.2	598
ss–k	0.0	527		
ss–ro			0.2	598

to the right, there is a very little crossing-over. Between this region and spineless, to the left, there is also a little. It seems probable that in the region to the left of spineless, crossing-over is very infrequent until a point about a unit to the right of pink is reached. Then to the left of this point the crossing-over is of normal frequency, or is even slightly high. The point of sudden transition from abnormally low to "standard" probably corresponds to the mid-point of the V-shaped chromosome.

THE LOCUS OF CIII.

The locus of CIII is in the region of its greatest effectiveness. Muller found, by testing the rare cross-overs, that the locus of CIII is situated to the right of kidney and to the left of liiia. The location at 75 has been chosen arbitrarily as near the middle of the section containing CIII.

HOMOZYGOUS CIII.

The data bearing upon the effect of homozygous CIII upon crossing-over is summarized in table 54. The significant feature is that in the homozygous

TABLE 54.—*Summary of recombination data, homozygous* CIII.

Loci (alphabetical).	Primary data.		Secondary data.	
	Per cent.	Numbers.	Per cent.	Numbers.
cr-III–e	41.3	889		
D–dw			6.6	1,778
D–ma	6.6	1,778		
ma–dw	0.0	1,778		
p–e	42.7	578		
se–p	27.2	136		
ss–e	41.6	445		

form, crossing-over in the right limb is as high as, or higher than, standard, and crossing-over in the left limb is likewise standard or higher.

USE OF CIII LIIIA IN BALANCING STOCKS.

Muller found that the pure-breeding stock of Beaded is one that carries in one third-chromosome a dominant Beaded gene that is lethal when homozygous, and in the other third-chromosome a recessive gene that is also lethal when homozygous. The CIII prevents crossing-over between the loci for the lethal and for Beaded, and consequently only two types of gametes are produced, viz, Bd and CIII liiia. By breeding together such flies, three kinds of zygotes are produced, the two homozygotes and a class twice as large (half the zygotes) heterozygous for both genes. Only the heterozygotes that are like the parents survive, and consequently the stock breeds true without selection.

The CIII liiia chromosome has been extensively used in balancing other dominants that are lethal when homozygous, e. g., Deformed, Delta, Hairless, Minute, and Minute-g, besides several complex stocks.

ROUGH (ro).

In working with the mutant Truncate-wing, Muller found in the stock (June 1913) a mutant with a "rough" eye, like that of the mutant morula (Altenburg and Muller, Genetics, January 1920, pp. 1–59). The ommatidia are variable in size and shape, and are crowded together in irregular rows. The "rough" eye is slightly smaller and narrower than normal, and slightly darker in color. The extent of roughening is very constant, and is so that in no case is there any doubt of the classification. The separations can be carried out very rapidly.

"Rough" was found to be recessive and not sex-linked. In crosses to third-chromosome recessives, rough gave typical 2 : 1 : 1 : 0 ratios that showed that its gene is in the third-chromosome. Muller combined rough with other

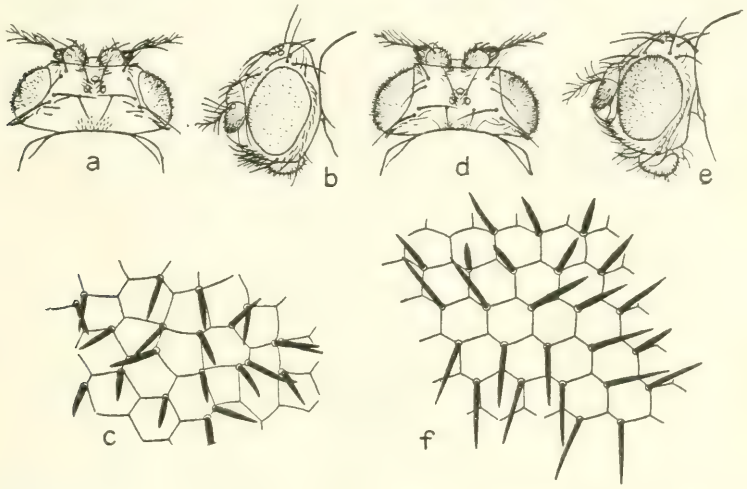


FIG. 9.—Rough eye. (*a*) top view; (*b*) side view; (*c*) enlarged view showing irregular arrangement of facets and of eye-bristles; (*d, e, f*) corresponding views of wild-type.

third-chromosome recessives, e. g., sepia or peach and spineless kidney sooty. In the course of this work it became apparent that the locus of rough is to the right of these others by a considerable interval. Back-crosses showed that there is about 20 per cent of recombination for sooty and rough. (Am. Nat., 1916, p. 217). The sooty rough recombination per cent is among the most constant known. The locus of rough as calculated from the total available data (see table 2) is 20.4 units to the right of ebony, or 91.1 units to the right of roughoid. Rough was for several years the "farthest right" workable mutant in the third chromosome. On account of its favorable position, normal viability, and its other excellent characteristics, rough is one of the most valuable and most used mutants in this chromosome.

DEFORMED (Df).

While working at Cambridge, England, in July 1913, Miss Eleth Cattell found a mutant similar in appearance to kidney. In extreme specimens the eyes were very small and were set far back on the head, which had a triangular shape in consequence. Miss Cattell found that the mutant was dominant.

The stock did not breed true, from which it is apparent that the homozygous form is lethal, or perhaps sterile. By crosses to pink she found that the locus of Deformed is in the third-chromosome.

Later, Muller made crosses with Deformed and determined its locus at about 3 units to the left of pink (see Am. Nat., 1916, p. 217). He found, however, that the character fluctuated greatly in amount of development, and overlapped the normal even to a greater extent than kidney. This fact made the mutant practically useless, except in very special work.

Muller used a Deformed pink spineless stock in crosses to Beaded (see Genetics, September 1918, p. 433 ff.), from which crosses he obtained flies carrying Df p ss in one chromosome and Chi and a lethal in the other. These flies were bred together, and nearly all of the offspring were like the parents (balanced lethal stock), but a few flies were pink spineless with very Deformed

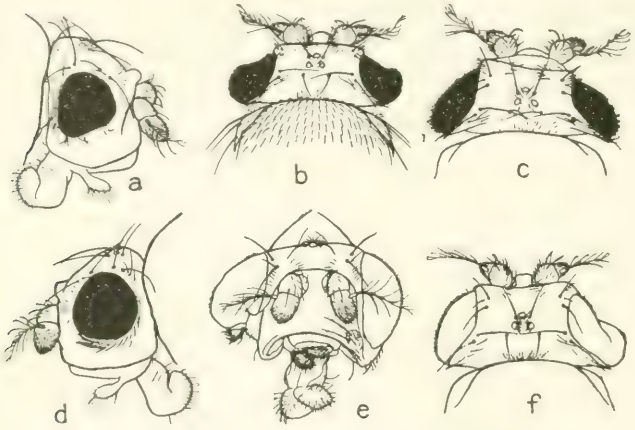


Fig. 10.—Deformed eye. (*a*, *b*, *c*, and *d*) side and top views of typical Deformed eyes. (*e* and *f*) front and top views of Deformed eyes of different grade on the two sides.

eyes. Evidently then, homozygous Deformed is generally lethal, but occasionally hatches as an extreme type with much Deformed eye and head. These homozygous Deformed flies were pale in color, dwarfed, and very weak, and had crumpled, flimsy wings. The males were completely sterile, and the females also were usually sterile, but occasionally produced a few offspring in crosses to not-deformed males. The crosses of homozygous Deformed males and females were entirely without progeny (see Muller, Genetics, September 1918, p. 462).

DEFORMED × DICHÆTE.

The data of Muller had made it seem probable that the locus of Deformed is about half-way between those of the mutants Dichæte and pink. Since these latter mutants are very excellent, there was not much need of a mutant located close to either. This fact, and the great fluctuation in the Deformed character, which overlapped the wild-type, accounts for the neglect of the mutant Deformed for about five years. With the accumulation of mutant loci in the neighborhood of pink, Deformed was taken up again as a possible

means of finding the order of these mutants. The usefulness of Deformed in this connection lies in its dominance, whereby quick, though not entirely reliable, determinations could be made of relative percentages of recombination.

Before Deformed could be used in this connection its own relation to the loci of Dichæte and of pink had to be determined more accurately. Accordingly a Dichæte × Deformed back-cross was carried out (table 55).

TABLE 55.—P_1. *Dichæte × Deformed; B. C., F_1 D Df ♀ × hairy ♂.*

Nov. 20, 1920.	Df	D Df	D	+
12,158.....	85	4	135	51
12,159.....	41	2	96	53
12,160.....	24	4	89	60
Total.....	150	10	320	164

There was 6.2 per cent of recombination for Dichæte and Deformed, a value considerably higher than expected, indeed higher than the usual Dichæte pink value.

HAIRY × DICHÆTE DEFORMED.

The above result led to the suspicion that the locus of Deformed might be to the left of that of Dichæte instead of to the right, and, accordingly, a hairy × Dichæte Deformed back-cross was carried out (table 56). The

TABLE 56.—P_1. *hairy × Dichæte Deformed; B. C., F_1 D Df ♀ × h ♂.*

Dec. 4, 1920.	D Df	h D Df	h Df	Df	h	+	D	h D
12,202.....	86	18	7	1	86	22	24	1
12,203.....	81	17	7	.....	102	18	33	4
12,234.....	52	23	11	2	73	18	31	5
Total.....	219	58	25	3	261	58	88	10

smallest class among the Deformed flies was the simple Deformed, which is thus the double-recombination class, showing that the order of loci is hairy Dichæte Deformed. The percentage of recombination for Dichæte and Deformed was 9.2, even higher than in the preceding experiment. The hairy Dichæte recombination per cent was 20.0 (on the basis of all the flies 17.9), which is a high value like that met with in certain other experiments in which hairy or roughoid had been involved.

DICHÆTE DEFORMED × SCARLET.

The locus of scarlet had been found to be about 3 units to the left of that of pink, and since the locus of Deformed was also to the left of pink (by an undetermined amount) the relation of Deformed to scarlet was next found

by a Dichæte Deformed × scarlet back-cross (table 57). The order of loci was shown to be Dichæte, scarlet, Deformed. There was, as before, 9.4 per cent of recombination for Dichæte Deformed and the normal value 3.2 for Dichæte scarlet (from all the flies). The scarlet Deformed recombination percentage, 9.4, is surprisingly high.

An interesting feature of this experiment was the occurrence of a double recombination, D st Df. No double recombinations had hitherto been met

TABLE 57.—*Dichæte Deformed × scarlet; B. C., F_1 D Df ♀ × st ♂.*

Dec. 17, 1920.	D Df	Df	st Df	D st Df	st	D st	D	+
12,244.....	38	.....	5	.....	115	5	54	1
12,245.....	57	1	4	1	85	4	35	1
Total.....	95	1	9	1	200	9	89	2

with for a section so short as that between Dichæte and Deformed. It is believed that the region about pink on the map represents a long section of chromosome in which there is relatively little crossing-over as compared with other regions of like actual length. Double recombination is believed to be a more direct function of chromosome distance than single recombination is.

DICHÆTE PINK × SCARLET DEFORMED.

The experiment with Dichæte, and especially that with scarlet, made the locus of Deformed seem much farther to the right than had been supposed, and brought into question the relation of Deformed to pink. The next experiment involved the four loci Dichæte, scarlet, Deformed, and pink (table 58).

TABLE 58.—*P_1, Dichæte pink × scarlet Deformed; B. C.,*

$$F_1 \frac{D}{+} \frac{+}{st} \frac{+}{Df} \frac{p}{+} \text{ ♀} \times st\ p\ \text{♂}.$$

Jan. 10, 1921.	st Df	D st Df	D Df	st Df p	D p	st	D st	p	st p	D
12,328.....	54	1	2	.....	146	94	5	3	4	5
12,329.....	42	4	5	.....	43	4	.....	1	2	.....
12,330.....	37	6	3	1	138	98	4	2	5	5
12,331.....	63	3	3	.....	151	71	2	3	5	6
12,332.....	88	3	5	.....	141	52	1	3	5	1
Total.....	284	17	18	1	619	319	12	12	21	17

The recombination percentages, on the basis of all the flies, are: D st 3.1, st p 4.3, D p 7.4. The D st value is normal, but the D p value is slightly above expectation. There was one st Df p fly, which shows that the locus of Df is really to the left of pink as was supposed, and that the two loci are very close together. The distance between Deformed and pink is about 0.3 unit, on the basis of the Deformed flies.

The parent of one of the cultures was an F_1 not-Deformed fly, as was shown by the progeny (table 59). The recombination per cents are much

TABLE 59.—*P_1, Dichæte pink × scarlet Deformed; B. C., F_1 D ♀ × st p ♂.*

Jan. 12, 1921.	Dp st	Dst p	D st p	D st p +
12,333.....	112 141	6 5	8 13	1 1

higher than in the sister cultures, and it seems probable that there is a linkage modifier involved.

DICHÆTE DEFORMED × PINK.

It seemed worth while to find the distance between Deformed and pink more accurately, since the two mutants were to be used as alternates in linkage experiments. Accordingly, a Dichæte Deformed male was crossed to a pink band female, and F_1 Dichæte Deformed females were out-crossed to pink males (table 60). There were 4 D Df p recombinations in the total of 694

TABLE 60.—*P_1, Dichæte Deformed × pink [band]; B. C., F_1 D Df ♀ × p ♂.*

Jan. 19, 1921.	D Df	Df	D Df p	p	D	D p	+
12,346.....	95	11	1	200	82	17	7
12,350.....	85	8	1	161	53	8	5
12,366.....	105	6	.....	198	111	19	7
12,367.....	115	7	2	214	87	19	13
12,368.....	103	11	.....	183	81	13	10
12,369.....	137	7	.....	223	91	21	8
Total.....	640	50	4	1,179	505	97	50

Deformed flies, or 0.6 per cent. The D p recombination per cent, on the basis of all the flies (2,525) was 8.0.

SCARLET DEFORMED PINK × SPINELESS.

A third experiment that gave the per cent of recombination for Deformed and pink was the back-cross of scarlet Deformed pink × spineless (table 61).

TABLE 61.—*P_1, scarlet Deformed pink × spineless; B. C., F_1 Df ♀ × st p ss ♂.*

Feb. 13, '21	st Df p	Df p	st Df ss	st Df p ss	Df p ss	st p	ss	st ss	p	st p ss	+	st	p ss
12,443....	112	8	.....	10	.....	112	157	12	4	11	22	1	1
12,444....	45	1	1	3	1	132	208	14	8	15	26	....	....
12,445....	58	....	.....	5	.....	78	132	7	6	9	19	....	1
12,446....	66	4	1	2	.....	124	154	12	3	12	15	1	2
Total..	281	13	2	20	1	446	651	45	21	47	82	2	4

There were 317 Deformed flies, of which 2, or 0.6 per cent, were recombinations for Deformed and pink.

DICHÆTE DEFORMED PINK × SCARLET.

A fourth experiment to determine more accurately the distance between the loci for Deformed and pink was the back-cross of Dichæte Deformed pink × scarlet (table 62). There were no recombinations for Deformed and pink in the 132 Deformed flies.

TABLE 62.—*P_1, Dichæte Deformed pink × scarlet: B. C., F_1 D Df ♀ × st p ♂.*

Feb. 23, 1921.	D Df p	Df p	st Df p	st	D p	D	st p	p
12,470	62	1	1	106	55	5	.....	.....
12,471	26	1	1	36	7	1	2	1
12,472	38	1	1	54	15	2	.....	.....
Total	126	3	3	196	77	8	2	1

The locus of Deformed, on the basis of the last four experiments, is 0.5 units to the left of that of pink.

SCARLET DEFORMED PINK × MAROON, F_2.

Muller had reported that homozygous Deformed, though weak and sterile, was sometimes able to live. A good opportunity of testing this question was offered by the possession of a scarlet Deformed pink stock, in which the Deformed was closely flanked by a recessive on each side. This combination was mated to maroon, and two F_2 cultures raised (table 63). The absence of

TABLE 63.—*P_1, scarlet Deformed pink × maroon; F_2, F_1 Df ♀ × F_1 Df ♂.*

March 3, 1921.	Df	st Df	Df ma	ma	+	st
12,529	112	9	4	84	51	3
12,532	114	5	2	81	14	.....
Total	226	14	6	165	65	3

scarlet Deformed pink flies in F_2 showed that no homozygous Deformed flies had lived. Treating Deformed as a lethal, the scarlet Deformed recombination per cent was 10.7 and the Deformed maroon 4.1.

EVALUATION OF DEFORMED.

A stock of scarlet Deformed pink maroon has been made up which will be valuable in locating the other mutants whose genes are situated in that region of the chromosome. The position of Deformed with reference to pink has

been rather accurately determined as 0.5 units to the left (47.5), and Deformed can serve as an alternate to pink in certain experiments. The special value of Deformed lies in its dominance, and its disadvantage is the fact that it overlaps the wild-type, so that only the Deformed flies can be used in calculations, and these none too confidently.

WHITE-HEAD.

In working with the stock of the third-chromosome recessive band, Morgan noticed (about August 1913) that many of the individuals had a silvery-white area on the top of the head. The grayish color normally present was practically absent from the region just outside of the group of ocelli and extending almost to the eye. Sometimes the character was restricted to a paler streak parallel to the margin of each eye. The character fluctuated greatly in extent, and for this reason almost no work was done with it. That its gene was in the third-chromosome seemed probable from the manner in which the characters white-head and band remained associated in crosses.

SOOTY (s).

(Plate 2, Figure 2.)

ORIGIN OF SOOTY.

In August 1913, Bar females from stock were mated by Sturtevant to ebony males from stock, and the F_1 Bar females were back-crossed to ebony males. Five cultures of this series gave the results reported earlier (Sturtevant, 1915) on the independence of Bar and ebony. A sixth culture, WH 113, containing 1 F_1 Bar female and 3 pink ebony males from stock, produced 134 Bar, 2 wild-type, 97 Bar ebony, 9 ebony. The sex-ratio was nearly 1 : 1, and the not-Bar offspring were 5 females, 3 males, 3 not recorded. The ratio of 231 Bar to 11 not-Bar, where equality was expected, led to tests of the offspring. Four Bar daughters were mated to males of different types. Three of these females gave the results expected from females heterozygous for Bar and for ebony, without any unusual ratios; and offspring of these females behaved in a similar orthodox fashion. The fourth Bar daughter of WH 113 was mated, in culture 2, to two white-eyed males from stock. The mating produced 86 Bar females, 74 Bar males, 0 not-Bar. A number of these 160 flies were mated to each other and to various stocks. No further unusual results were obtained, though the fact that some of the 160 proved to be heterozygous for ebony indicates that contamination was not responsible for the unusual results produced by WH 113 and 2. This curious case has never been explained. It is described here because of the results produced by culture 27, which contained a Bar male from 2 and a wild-type female of a stock collected at Harris, Minnesota, by Miss M. B. Stark. This culture produced 155 flies with the wild-type body-color, and 54 dark-colored specimens (the first "dark" were observed October 20, 1913). These dark flies were found to be heterozygous for ebony (from the father) and for a new allelomorph of ebony, called sooty. The Minnesota female was evidently heterozygous for sooty. The mutation probably occurred "in nature," as this

Minnesota stock came from fruit exposed outdoors and shipped to New York not earlier than September 20, 1913. The female used in culture 27 must have emerged on or before September 30, for No. 27 was made up on that date. She therefore came from an egg laid before the fruit was collected, or else laid by a "wild" female a few days after it was collected.

DESCRIPTION OF SOOTY.

The mutant sooty is the least extreme of the ebony allelomorphs. The trident-pattern on the thorax is sharp and about as dark as a well developed "with." The ground-color of the thorax and of the wings, legs, head, and abdomen is dusky. There is a sooty band bordering each cell of the wing as in black and ebony, but not as pronounced. The general color is also a little slower to develop than in the case of ebony or black, so that separations in very young flies are difficult. It is customary to return these young flies to the culture-bottle unclassified. By the time the next count is made they will have developed their full color.

The ebony-sooty compound is nearly as dark as ebony. Ebony and sooty are separable, but cultures containing the two primary allelomorphs and their compound are not classifiable.

CHROMOSOME AND LOCUS OF SOOTY.

By establishing the fact that sooty is an allelomorph of ebony, Sturtevant showed that the locus of sooty is in the third chromosome and in the position occupied by ebony. This position was thought to be to the right of pink by an amount that was uncertain because of the extreme variation in the amount of crossing-over.

Muller combined sooty with spineless and found that the locus of sooty was about 12 units to the right of spineless. Sooty is free from Ciii, and, primarily for this reason, has been used in linkage experiments instead of ebony.

EVALUATION OF SOOTY.

The viability of sooty is not noticeably different from that of wild flies, and is better than that of the other and more extreme ebony allelomorphs. The sooty character is distinct enough from the wild-type so that separations are certain, though not quite as rapid as desirable. There is another advantage in this slight degree of the sooty character; in general, slight characters interfere with the detections of other mutants affecting the same body regions or characteristics, and with the subsequent use of such mutants, to a correspondingly slight degree. The only other known third-chromosome body-color is band, the locus of which is so close to that of ebony that the two would not need to be used together.

The ebony locus has been one of the most valuable in the third chromosome. Since the discovery of the excellent dominant Hairless, the locus of which is only 1.2 units to the left of that of ebony, these two loci have been used interchangeably in experiments in which this region of the chromosome is to be followed.

DWARF (dw).

ORIGIN OF DWARF.

In order to determine the locus of the second-chromosome recessive "arc" by means of a back-cross, the double recessive arc speck was made up. Arc was crossed to speck; F_1 wild-type males and females were inbred; F_2 arcs were bred to F_2 specks; F_3 cross-over speck flies were inbred, giving arc speck doubles in F_4. In one of the F_3 mass-cultures (II–106) Bridges found 3 females that were strikingly small and that had a purplish eye-color (November 12, 1913). These dwarf females were sterile, but their normal brothers and sisters were bred together, and (in F_4) other dwarfs were produced.

MAROON BY REMUTATION.

In some of the F_4 cultures the purplish eye-color was present without the dwarf character. Tests of this eye-color showed that its locus was in the third chromosome (table 16), and that it was, in fact, maroon that had reappeared by a fresh mutation (see p. 60).

FEMALE-STERILITY OF DWARF.

Practically all of the dwarfs that appeared had maroon eyes. To rid the stock of the second-chromosome recessives, arc and speck, some of the maroon dwarf males were out-crossed to wild females, and after a few generations of selection, a maroon dwarf stock was maintained by breeding maroon dwarf males to wild-type heterozygous sisters. Many attempts were made to breed from dwarf females, but all of these failed. Subsequently, extensive tests were carried out by Miss Clara J. Lynch, and these tests showed that dwarf females are invariably completely sterile, and in fact do not lay eggs. This sterility holds for the dwarf females that overlap normal in size, so that it is a specific effect of the dwarf gene.

CHROMOSOME OF DWARF—MAROON DWARF CROSSING-OVER.

The fact that practically all of the dwarf flies that appeared in the F_3 and F_4 cultures referred to above were at the same time maroon-eyed, showed, without further test, that the locus of dwarf is in the third-chromosome.

TABLE 64.—*P_1, maroon dwarf ♂ × wild ♀; B. C., F_1 + ♀ × ma dw ♂ (heterozygous CIII).*

Nov. 4, 1914.	ma dw	+	ma	dw
700	52	112	3	5
701	89	170	1	5
702	54	180	1	3
704	148	226	1	1
705	77	147	2	2
706	135	244	5	0
Total	555	1079	13	16

Furthermore, the possession of the double recessive maroon dwarf made it possible to carry out a back-cross at once, and thereby obtain one of the two cross-over values that are necessary in order to determine the locus of a mutation (table 64). Some little difficulty was found in the classification of dwarf, so that the results of table 64 are to be regarded as only approximately correct; however, it is certain that there was very little crossing-over between the loci of maroon and dwarf. Only 1.7 per cent of the back-cross flies were recombinations.

THE USE OF DICHÆTE AND HAIRLESS IN LOCATING DWARF.

The viability of dwarf, in the maroon dwarf back-cross, was not at all good. The maroon dwarf class was only about half as large as the wild-type complementary class that is expected to be of the same size. The eye-color maroon is known to be of normal viability. Because of this heavy mortality little work was done with the dwarf mutant until after the discovery of the two excellent third-chromosome dominants Dichæte and Hairless. The locus of Dichæte was quite near the left end of the third-chromosome as then known (about 12 units to the right of sepia), while the locus of Hairless was not far to the right of the supposed middle of the chromosome (about 11 units to the right of spineless). By means of these two dominants, favorably placed and separated by about 29 units (DH recombination per cent=25.6), it was possible to find the location of a new third-chromosome character with a single back-cross test. For this purpose a stock had been made up in which the two dominants were carried in the same member of the third-chromosome pair. In order to determine the locus of dwarf by this method, a maroon dwarf male was out-crossed to such a Dichæte Hairless female, and F_1 Dichæte Hairless females were back-crossed to maroon dwarf males. The presence of the maroon was an additional advantage in placing both maroon and dwarf in their relation to each other and to Dichæte and Hairless, the primary bases of reference.

THE PRESENCE OF CIII IN THE MAROON DWARF CHROMOSOME.

The result of the above back-cross (table 18) was surprising, for instead of the expected 25 per cent of recombination for Dichæte and Hairless, only 6.1 per cent was observed. Nearly all of this recombination (5.9) was due to crossing-over between Dichæte and maroon, with only a very little (0.2) between maroon and dwarf, and none at all between dwarf and Hairless. This situation was at once seen to be parallel to that described by Sturtevant and by Muller for crossing-over in flies heterozygous for the cross-over variation CIII. It had been found that in heterozygous CIII there is only about 1 or 2 per cent of recombination for pink and ebony, and that most of this is in the region immediately adjacent to pink. Normally the recombination per cent for pink ebony is about 20. The Dichæte Hairless stock was known to be free from CIII, and the variation must therefore have been introduced through the maroon dwarf stock. The presence of a CIII in the maroon dwarf stock was not demonstrated until much later, when a maroon dwarf male was crossed to a spineless white-ocelli female, and back-cross tests of the heterozygous

females showed that there was no recombination for spineless and white-ocelli instead of the 17.5 per cent that indicates normal crossing-over.

The occurrence of two maroon not-dwarf Hairless individuals in the cross of table 18 proved that the locus of dwarf is to the right of that maroon, which is itself between Dichæte and Hairless and about 5 units to the right of Dichæte. The length of the interval between maroon and dwarf could not be determined from the data of the above cross, as CIII was present. As no crossing-over was detected between the locus of dwarf and that of Hairless, and as the locus of CIII is probably not far from that of Hairless, there was almost no chance of obtaining dwarf flies free from CIII. In the absence of such flies the normal crossing-over relations of dwarf could not be determined by direct experiment.

LINKAGE RELATIONS OF DICHÆTE MAROON AND DWARF IN HOMOZYGOUS CIII.

A way out of this difficulty was offered by testing the amount of crossing-over in the presence of homozygous CIII, under which condition the amount of crossing-over between pink and ebony is raised much above normal. A maroon dwarf CIII male was outcrossed to a female carrying Dichæte and CIII in the same chromosome. The F_1 Dichæte females were then back-crossed by maroon dwarf males (table 19).

In spite of the freer crossing-over due to homozygous CIII, no recombinations for maroon and dwarf were observed among the 1,778 flies. This means that the locus of dwarf is very close to that of maroon, probably within half a unit to the right of maroon. There was 6.6 per cent of recombination for Dichæte and maroon, which is practically the same as in the heterozygous CIII experiment. It was known that the reduction of crossing-over due to heterozygous CIII does not extend to the left of pink; it is therefore probable that the rise due to homozygous CIII likewise does not extend to the left of pink. As the locus of maroon is very close to that of pink, the Dichæte maroon value should be practically the same in homozygous and in heterozygous CIII, as was observed to be the case.

ORIGIN OF THE CIII OF THE MAROON DWARF STOCK.

There is no known connection between the maroon dwarf stock and any other stock containing CIII, but it is probable that there is such a connection. It seems likely that the CIII mutation occurred very early in the *Drosophila* work, before the Beaded mutation (May 1910), and that the ebony mutation took place in the chromosome carrying CIII. Further, this CIII chromosome became distributed very widely by crosses of Beaded, of Truncate, and others of the many mutations that arose in Beaded stock or in stocks descended from it. Finally, the mutations maroon and dwarf arose successively in such a CIII chromosome.

WEIGHT CURVES OF DWARF FLIES.

In working with the mutant "dwarf-b" (see p. 228) it was noticed that the separation of dwarf-b from non-dwarf-b sibs became considerably easier in the later counts of the cultures. This same condition was present, but to

a less extent, in the cultures of dwarf and non-dwarf. Determinations of the weights of flies, and of the way in which this weight changes with the age of the culture, were made for both dwarfs and dwarf-b's. In the case of dwarf, a maroon dwarf male was crossed to a spineless white-ocelli female and the F_1 wild-type females were back-crossed to maroon dwarf males. Since the amount of recombination for maroon and dwarf is probably less than 1

TABLE 65.—*Weights in milligrams of dwarfs (above) and wild-type sibs (below); days are class centers.*

Days	dw	mg.	dw	mg.	dw	mg.	dw	mg.	dw	mg.	dw	mg.	dw	mg.
	1		3		5		7.5		10		12			
12,062	44	39.5	40	31.8	29	19.0	23	12.0	21	10.5	18	8.4	.....	
12,063	22	22.0	29	22.0	30	18.2	24	13.3	13	6.4	14	6.7	.....	
12,064	45	44.0	37	30.6	27	18.5	28	16.2	28	13.8	16	7.7	.....	
Days.	.5		2		4.5		7		9		11.5		14.5	
12,126	6	5.7	21	15.5	20	14.8	15	7.0	7	4.4	15	5.3	14	6.4
12,127	2	2.3	29	25.4	16	11.2	11	5.3	13	7.4	15	5.7	16	8.0
12,128	17	12.7	27	20.7	16	12.0	14	6.9	21	10.3	7	3.5	13	5.9
12,129	12	9.8	28	20.5	16	10.7	13	7.0	16	8.0	15	8.0	10	5.4
Totals	148	136.0	211	166.5	154	104.4	128	67.7	119	60.8	100	45.3	53	25.7
Av. days	.8		2.6		4.7		7.2		9.6		11.7		14.5	
Av. weight	.92		.73		.68		.53		.51		.45		.48	
	+	mg.	+	mg.	+	mg.	+	mg.	+	mg.	+	mg.	+	mg.
12,062	60	63.0	46	47.6	31	28.0	30	25.0	28	20.7	14	9.4	.....	
12,063	46	48.6	39	39.4	33	28.4	32	28.0	16	12.0	18	11.2	.....	
12,064	48	52.0	50	50.6	39	36.4	30	28.7	13	12.3	11	13.7	.....	
12,126	17	16.3	25	20.8	18	12.1	23	15.2	12	6.7	11	5.4	13	8.4
12,127	13	12.2	37	34.5	20	16.8	19	12.7	17	11.0	15	7.9	9	5.8
12,128	17	22.9	42	40.9	21	17.1	19	13.1	12	7.8	17	9.3	14	7.4
12,129	16	16.1	39	37.2	33	29.7	19	14.1	12	8.1	17	9.7	14	8.9
Totals	217	231.1	278	271.0	195	168.5	172	136.8	110	78.6	103	66.6	50	30.5
Av. weight	1.06		.97		.86		.79		.71		.65		.61	

per cent. only negligible error is involved in classifying all maroon flies as dwarfs and all non-maroon flies as non-dwarfs.

The flies that hatched in each two-day interval were separated into dwarfs (maroon) and non-dwarfs (non-maroon) and the two kinds were weighed separately (table 65). Of the 7 cultures, 3 were raised in one generation and the other 4 in the next generation. This procedure gave greater variety of cultural conditions and greater generality to the results. The two sets of data were combined and were reduced to average weight per fly in milligrams.

The curves of average weights for the dwarfs and the non-dwarfs in relation to age of the culture are given in figure 36, page 231. The initial weight of the dwarf flies was 0.92 mg. as compared with 1.06 for the non-dwarf. Both weights decreased continuously throughout the course of the cultures, the relative difference being nearly constant. The average weight of the non-dwarfs was 0.87 mg., and of the dwarfs 0.66 mg. or 76 per cent of the normal weight. It takes slightly more than half a million of the not-dwarf sibs to weigh a pound, while in the later counts it would require approximately a million dwarfs to weigh a pound.

The weight of the not-dwarf sibs was considerably less than that of not-dwarf-b sibs, and it is possible that this means that the dwarf gene is semi-dominant.

EVALUATION OF DWARF.

The present usefulness of dwarf is largely in balancing stocks through the presence of Cin combined with female sterility. The degree of overlap with the wild-type is large enough, especially in the early counts of cultures, to make the linkage data not very reliable.

SPREAD (sd).

(Figure 11.)

ORIGIN OF SPREAD.

The mutant spread-wings was found by Dexter in November 1913 in the F_2 from the cross of Beaded (not of the pure-breeding stock) to vestigial.

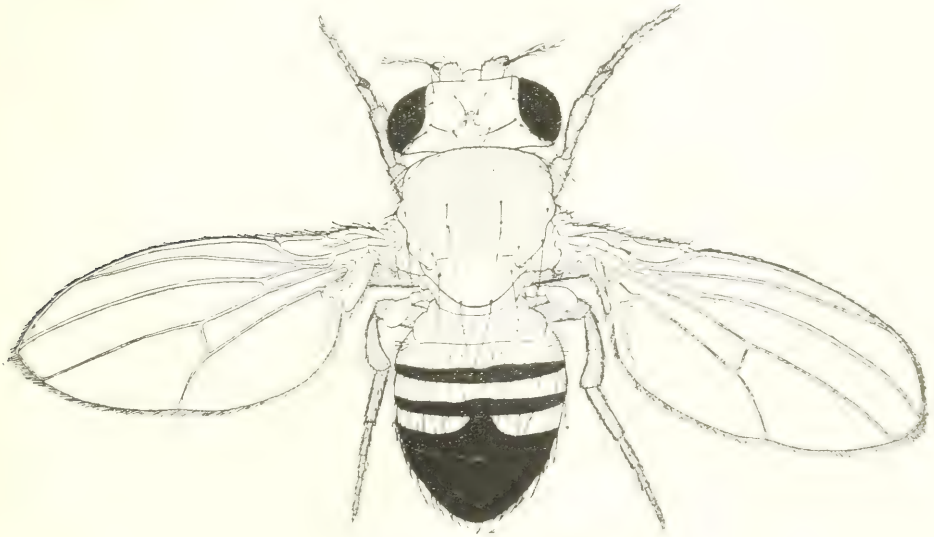


Fig. 11.—Spread wings.

(See Am. Nat., Dec. 1914, p. 755, for Dexter's account of spread.) Several of the pairs of F_1 wild-type flies produced spread among the F_2 flies, from which it is apparent that one of the P_1 flies was heterozygous for the recessive

gene for spread. None of the spread flies were Beaded. Spread flies were mated together, and a pure-breeding stock free from Beaded was obtained at once.

DESCRIPTION OF SPREAD.

The wings of the flies of the spread stock are held out at right angles to the body. The texture of the wings is slightly weaker than normal, and the wings are liable to become bedraggled because of their thinness and position. There is little variation in the angle of the wings to the body, and the spread flies are thus easily and completely separable from the wild-type. The viability is good.

CHROMOSOME OF SPREAD.

Spread males were outcrossed by Dexter to females of the double mutant type black pink. In F_2 the spread reappeared in about a quarter of the individuals, none of the spread flies being at the same time pink. This proved that the gene for spread is in the third chromosome. F_2 pink males and females were bred together, but no pink spread appeared in F_3. Pink spread flies were found later in the spread stock, showing that crossing-over had occurred, but had not been detected.

PRESENCE OF CIII IN SPREAD.

Nothing was known of the location of spread within the third chromosome when Muller began to get multiple stocks ready for his multiple heterozygote tests in the spring of 1915. An unexpected difficulty was met with in that there was found to be almost no crossing-over in the females heterozygous for spread and for other third-chromosome mutants. Further analysis of this situation, together with similar cases with ebony and with Beaded, led to the hypothesis of a dominant cross-over variation due to a gene carried in the third chromosome (see p. 89 for a full account of CIII).

The spread stock appeared to be homozygous for CIII. This fact throws considerable light upon the origin of spread. The Beaded flies of the stock from which the mating of Beaded to vestigial was started, carried the dominant gene for Beaded in one member of the third-chromosome pair, and in the other the cross-over variation CIII. The mutation to spread occurred in the CIII–chromosome, some time previous to the above mating. It will be remembered that the pure-breeding stock of Beaded had also resulted from the occurrence of the lethal mutation IIIIa in the CIII–chromosome of this same Beaded stock (see p. 43). All flies homozygous for IIIIa die; likewise all flies homozygous for the Beaded gene die; and the presence of CIII prevents crossing-over between these two loci, so that the inbred Beaded stock does not give rise to any flies other than the parental type, viz: $\frac{\text{Bd}\cdot}{\text{CIII}\quad \text{IIIIa}}$

LOCUS OF SPREAD, HETEROZYGOUS CIII.

Because of the presence of CIII in spread, it was not possible to find the locus of spread in the third chromosome by direct tests. As in the parallel case of dwarf, the locus was first found in relation to certain genes in the presence of heterozygous CIII. A spread male was crossed by Muller to a peple

(p^p ss k e^s ro) female, and F_1 females were back-crossed to peple males. For the region to the right of pink (pink to rough) there was only a little over 1 per cent of recombination. One sooty rough fly appeared, and tests showed that it carried neither spread nor Ciii; that is, the loci of both these mutants lie to the right of that of kidney (Am. Nat., 1916, p. 219). Kidney itself lies about 7 units to the left of ebony.

LOCUS OF SPREAD, HOMOZYGOUS CIII.

The stock of pink ebony was known to be homozygous for Ciii. Muller then made crosses between this stock and the spread stock, by means of which he determined that in homozygous Ciii the locus of spread is roughly two-thirds of the way from pink to ebony. The interval between pink and ebony is normally 22.7, so that the locus of spread is about 7 units to the left of that of ebony. But the test in heterozygous Ciii showed that the locus of spread is to the right of that of kidney (roughly 64), so that we are now able to place spread very close to the right of kidney, at about 65 (Am. Nat., 1916, p. 220).

EVALUATION OF SPREAD.

The stock of spread died out before the mutant had been used extensively. A valuable means of investigating linkage conditions in the presence of homozygous Ciii was lost thereby.

LETHAL–III C (LETHAL IN PINK BEADED).

In analyzing the constitution of a stock that was apparently breeding true for the character Beaded, Dexter raised some F_2 cultures from the cross of Beaded × pink ebony Ciii (see vol. 48, p. 737, Am. Nat., 1914). In F_2 a few pink Beaded flies occurred as a result of crossing-over, and from these Dexter secured a pink Beaded stock that bred as true to the Beaded character as had the original stock (November 1913). In the light of later knowledge of Beaded, the fact of a homogeneous stock of Beaded means that in the opposite third-chromosome there was a balancing lethal. This opposite chromosome therefore carried pink ebony Ciii and a new lethal situated to the right of pink. In the formation of the gametes of the fly with Beaded in one third-chromosome and Ciii liiia in the other, the Beaded and the original lethal (liiia) went into different gametes. When such a fly was out-crossed to pink ebony Ciii the Beaded offspring were entirely free from the original lethal. These Beaded F_1 flies carried Beaded in one chromosome and pink ebony Ciii in the homolog. Some of the F_1 flies carried a new lethal in the pink ebony Ciii chromosome. Presumably the P_1 pink ebony had been heterozygous for the lethal, and it had been present undetected in the pink ebony stock. The pink Beaded balanced stock was maintained by Dexter for a year or more, and was used in several crosses.

LETHAL–III D (LETHAL WITH PINK).

In the early work with pink, Morgan had found several cultures in which the number of pink flies was far behind the expected 25 per cent. These cultures were mainly mass-cultures, and it was thought that some mistake

had been made in the mating, so that the cultures were of mixed parentage. For this reason Miss Moody was asked to repeat the crosses, using pair-matings throughout. The cultures raised by Miss Moody were of two kinds; about a quarter produced only a small proportion of pinks, while the others gave the normal 3:1 ratio (Morgan, 1912).

An attempt was made to explain these new results on the ground that pink hatches somewhat later than the wild-type flies. Mr. Liff (Liff, 1915) made a careful study of this phase of the problem, and found that while it was indeed true that pink hatched later than wild-type and that this difference might be emphasized by excessive wetness or excessive dryness of cultural conditions, yet the amount of this delay is totally inadequate to explain the results of Morgan and Moody. The many cultures raised by Liff were remarkably free from aberrant ratios. Only five cultures were encountered that appeared to be significantly different from the usual result. By this time the hypothesis of lethal genes had been developed (Rawls, 1913, Morgan, 1912), and it was suggested (by Muller) that these few aberrant ratios of Liff's were due to a recessive autosomal lethal linked to pink. It now appears that this is the true explanation, though the experiments of Liff were not suitable to prove the point. The recent work of Muller, Bridges, and Sturtevant has furnished many instances of such autosomal lethals and has developed methods of handling them.

Four of the aberrant cultures of Liff (Liff, 1915, p. 118, cultures raised about December 1913) had every appearance of being of the same type. Together they gave a total of 2391 wild-type and 310 pink flies, from which the per cent of recombination can be calculated as 32.7. It is not known whether the locus of IIIId is to the left or to the right of pink.

The other aberrant culture raised by Liff can not be explained on a simple lethal basis. This back-cross culture (No. b, table XI, Liff, 1915) gave 10 pink and 586 wild-type flies, which is a proportion of pink far below the minimum value of 33.3 per cent that should obtain in cultures of the back-cross type. For this case there are some 8 or 10 possible explanations (including duplication, specific modifications of pink to wild-type, partial and dependent dominant lethals, etc.), but in the absence of data that would furnish tests no detailed discussion would be worth while.

BEADED-INTENSIFIER-III.

In analyzing the constitution of Beaded, Dexter out-crossed Beaded to various mutant stocks, and made counts of the percentage of Beaded that occurred in F_1. This percentage was variable, but most of the cultures gave between 15 and 30 per cent of Beaded. In F_2 of the cross of pink ebony to Beaded some pink Beaded flies occurred, and from these was made a stock of pink Beaded. When pink Beaded flies of this stock were out-crossed (about December 1913) to other stocks such as white, black, and wild, there were produced in F_1 markedly fewer Beadeds than had been given in like out-crosses of the parent Beaded stock. This fact is interpreted as due to the absence from the pink Beaded stock of a dominant intensifier present in the selected Beaded stock, but removed by crossing-over between pink and Beaded.

The locus of the intensifier is thus to the left of the point near pink at which the effect of CIII ends.

SPINELESS (ss).

(Figure 12; Plate 3, Figures 2 and 3.)

ORIGIN OF SPINELESS.

In working with cream–II, Bridges had found a new type of abdomen called patched (see p. 241, Carnegie Publication No. 278). At the same time, Morgan was working with a similar character called broken-bands. A stock

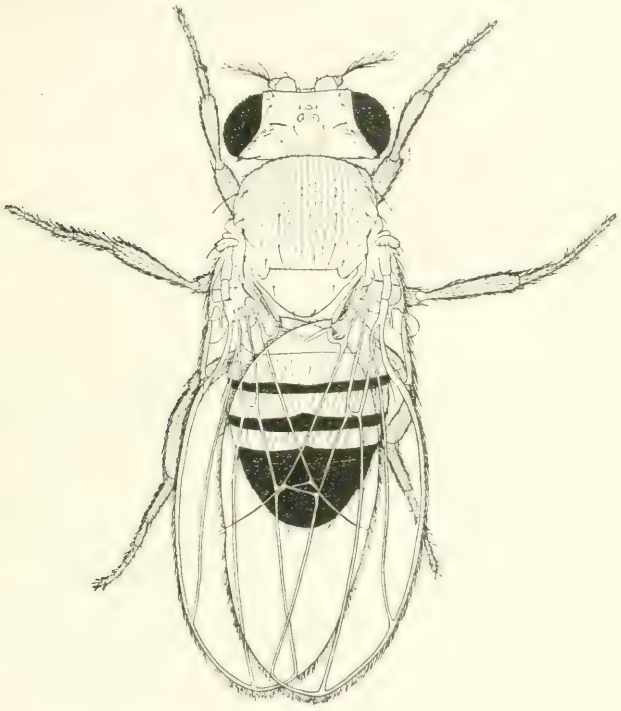


FIG. 12.—Spineless.

of broken-bands was given to Bridges for comparison of the two kinds of abnormal abdomen. In the stock of broken-bands were found a few flies in which all the large bristles, normally present on the head, thorax and abdomen, were greatly reduced in size, being scarcely larger than the microchæte (No. 68, January 3, 1914).

In this respect the new character resembled morula (see p. 230, Carnegie Publication No. 278), which was the first mutant in which the bristles were affected. The mutant morula is characterized also by the moruloid nature of the eye—a disarrangement and crowding of the facets. It was thought that the new character "spineless" might be related to morula, perhaps a "fractionation product." At this same time "facet" was found (see Carnegie

Publication No. 237, p. 76) in which the eyes are moruloid but the bristles normal. This highly suggestive situation lost its interest when it was found that the genes of the three characters facet, morula, and spineless were in different chromosomes, the first, second, and third, and that the crosses between the mutants produced wild-type flies in F_1.

THE CHROMOSOME OF SPINELESS.

It had seemed probable that spineless was allelomorphic to morula and therefore in the second chromosome, and accordingly spineless was crossed to the second-chromosome recessive black. The F_1 flies were wild-types, showing that the spineless was recessive, as had been suspected from the manner of its occurrence in the original broken-band culture. In F_2 (table 66)

TABLE 66.—P_1, *black* ♂ × *spineless* ♀; F_2, F_1 + ♀ × F_1 + ♂.

July 27, 1914.	+	b	ss	b ss
348.....	259	81	71	26
349.....	178	45	38	15
350.....	257	97	95	21
Total.....	694	223	204	62

the spineless reappeared in about a quarter of the individuals, both males and females, from which it follows that the character is not sex-linked. That it is not a member of the second group was shown also by the 9 : 3 : 3 : 1 ratio, including the double recessive black spineless.

Most of the spineless flies of this F_2 were seen to possess the character white-ocelli (see p. 67). This probably does not mean a new appearance of the white-ocellar mutation, but that the spineless mutant occurred in a stock derived from the black stock in which white-ocelli was discovered.

Since the gene for spineless was thus shown not to be in the second chromosome, it was almost certainly in the third, and accordingly work was started at once to obtain double recessives by which its position in the third chromosome could be determined. Spineless was crossed to spread (recently discovered by Dexter), to peach, and to sepia. F_2 cultures were counted, and these gave the expected 2 : 1 : 1 : 0 ratios (tables 67, 68, and 69).

From the peach by spineless F_2, peach and spineless flies were mated, and in F_3 peach spineless was obtained. A stock of peach spineless was turned

TABLE 67.—P_1, *spineless* ♀ × *spread* ♂; F_2, F_1 + ♀ × F_1 + ♂.

Aug. 24, 1914.	+	ss	sd	ss sd
561.....	225	111	94	0
562.....	54	24	28	0
Total.....	279	135	122	0

into the stock-room, and was used by Muller in making up the "peple" stock. Likewise a stock of sepia spineless was derived in F_4 from the cross of sepia by spineless, and this was used in making the "seple" stock.

TABLE 68.—P_1, *peach* ♂ × *spineless* ♀; F_2, F_1 + ♀ × F_1 + ♂.

Aug. 27, 1914.	+	p[p]	ss	p[p] ss
563.....	189	80	57	0

TABLE 69.—P_1, *sepia* ♀ × *spineless* ♂; F_2, F_1 + ♀ × F_1 + ♂.

Aug. 25, 1914.	+	se	ss	se ss
564.....	55	33	29	0

In the F_2 out of spineless by spread, yellow appeared by remutation, and the first mosaic involving an autosomal character was found (see Carnegie Publication No. 278, p. 26). In the spineless by sepia F_2 appeared the third-chromosome character giant, and also an eye-color mutation.

LOCUS OF SPINELESS.

From the first, the stocks involving spineless were used very extensively. The crossing-over data indicated that the locus of spineless is about 10.5 units to the right of pink and 12.2 to the left of ebony (table 2).

SEPIA SOOTY ROUGH × SPINELESS.

A back-cross of sepia sooty rough × spineless gave 12.3 per cent of recombination for spineless sooty (table 70).

TABLE 70.—P_1, *sepia sooty rough* × *spineless*; *B. C.*, F_1 + ♀ × *se ss e[s] ro* ♂.

March 24, 1920	se	ss	se	ss	se	ss	se	ss	se	ss	se	ss	se	ss	se	ss
			ss			ss		ss	ss		ss			ss	ss	
	e[s]			e[s]	e[s]		e[s]		e[s]	+		e[s]	ro		e[s]	
	ro			ro	ro		ro		ro			ro			ro	
11,601.....	93	112	60	36	13	28	45	38	4	6	5	10	1	2	1	1
11,602.....	81	91	33	28	12	19	40	35	6	3	11	15	. .	1	. .	1
11,603.....	91	87	25	25	15	20	38	21	6	6	7	4	1	1	.	1
11,605.....	110	99	42	47	23	15	43	23	5	3	8	13	2	. .	2	1
Total....	375	389	160	136	63	82	166	117	21	18	31	42	4	4	3	4

EVALUATION OF SPINELESS.

The recombination percentages obtained for spineless and the various loci to the right of spineless are surprisingly uniform in the different experiments. The mean value for spineless ebony is 12.2. The values obtained with pink

and with Dichæte are among the most variable found for the third chromosome. The explanation of these differences seems to be that the region to the right of and including spineless lies within one limb of the V-shaped III-chromosome, while the region including Dichæte and pink lies within the other limb. (For further consideration of this question of the mid-point, see p. 28.) All crossing-over experiments in which this mid-point is bridged seem to be especially variable in the amount of crossing-over. The mid-point lies between pink and spineless, probably much nearer to pink, and this mid-region is especially sensitive not only to effects by genetic factors, but also to environmental factors, such as age and temperature. The length of the interval between pink and spineless is therefore uncertain. The most probable value seems to be 10.5 units.

The only other usable mutant in this region just to the right of the mid-point is bithorax, which is somewhat poor in viability and also in certainty of identification.

The character spineless is exceptionally easy to classify, and the separation from the wild-type is complete. There is almost no fluctuation in the amount of the development of the bristles. In viability, fertility, and productivity, spineless is normal. Spineless can be used with all other third-chromosome characters (including hairy, Dichæte, and Hairless) except Minute.

CREAM–III (cr III).*

(Plate 1, Figures 4, 5, 7, and 8.)

ORIGIN OF CREAM-III.

In a culture that was part of an experiment on lethal 1 b (Carnegie Publication No. 237, p. 76), Miss E. M. Wallace found (February 27, 1914) a few females which were much lighter in color than their white-eosin sisters. These flies were given to Bridges, who carried out the following experiments:

INHERITANCE OF CREAM-III.

One of these females was out-crossed to an eosin male of pure stock (Jour. Exp. Zool., July 1919, pp. 338 and 341). The F_1 males (table 71) showed

TABLE 71.—*F_1 and F_2 from outcross of new cream (cream III) ♀ to eosin ♂.*

March 9, 1914.	w-w^e ♀	w^e ♀	y w ♂	w^e ♂	y w^e ♂	w ♂
92.....	25	21	15	24	..	..
March 26, 1914.	w^e ♀	w^e ♂	cr (III) ♀		cr (III) ♂	
136.....	91	92	22		21	

* An account of cream–III, practically the same as here given, has been published as a part of an account of the eight "Specific modifiers of eosin eye-color in *Drosophila melanogaster*," by Calvin B. Bridges, Jour. Exp. Zool., July, 1919, pp. 337–384.

that this female had carried the sex-linked characters yellow and white in one X and eosin in the other, this being the condition expected from her parentage. The "cream" was seen to be recessive (since all the F_1 flies were standard in color) and not sex-linked, as it did not appear in the sons of the cream mother.

A mating was made between an F_1 eosin female and an F_1 eosin male, since by this means both yellow and white could be eliminated from the experiment. In F_2 the cream-color reappeared in numbers rather fewer than the expected quarter (table 71).

From the cream males and females of culture 136, a pure stock of the double-recessive eosin cream was made up.

CHROMOSOME OF CREAM-III.

In order to find out in which chromosome the gene of this cream lies, a second cream female (found February 28, in the same culture as the first)

TABLE 72.—*F_1 and F_2 from outcross of cream* (III) ♀ *to eosin black* ♂.

March 18, 1914.	w-w^e ♀	w^e ♀	y w ♂	w^e ♂	y w^e ♂	w ♂
121.....	52	61	35	58	..	3
April 4, 1914.	w^e	w^e b	cr III		b cr III	
159.....	118	45	56		15	
160.....	136	49	41		20	
Total.....	254	94	97		35	

was mated to an eosin black male of the stock that had been made up for testing cream–II. Two F_1 pairs (eosin female × eosin male) gave a 9 : 3 : 3 : 1 ratio (table 72), which proved that the gene for this cream was not in the second chromosome.

The fact that this gene was found not to be in chromosome II made it probable that it would be in chromosome III, since chromosome IV is very small. To test this point, a cream female from the pure stock descended from culture 136 was mated to an eosin ebony male from the stock that had been used in testing cream–II. Three pairs of eosin F_1 flies gave the approximation to a 2 : 1 : 1 : 0 ratio observed in table 73. The absence of the double

TABLE 73.—*F_2 from outcross of cream* (III) ♀ *to eosin ebony* ♂.

Aug. 3, 1914.	w^e	cr-III	w^e e	cr-III e
361.....	85	45	47	0
363.....	106	39	42	0
371.....	116	68	41	0
Total.....	307	152	130	0

recessive cream ebony proved that this cream was in chromosome–III, and it was accordingly called cream–III,* the Roman III being the symbol for the third-chromosome.

CREAM-III EBONY CROSSING-OVER.

It was decided to find approximately the locus of cream–III in the third-chromosome. To do this it was necessary to obtain the amount of crossing-over between cream–III and two other genes whose loci were already known. The best method of finding the amount of crossing-over consists in mating a multiple heterozygote to the corresponding multiple recessive. The double recessive cream–III ebony was made up by mating to each other the eosin ebony and the cream–III flies of F_2 (culture 361). Nearly all of these matings gave only eosin flies in F_3, but one pair gave half of the flies eosin and the other half eosin ebony. These eosin ebony flies must have been heterozygous for the cream gene, since their father was homozygous cream. When these F_3 eosin ebony flies were bred together, a quarter of their offspring (F_4) were cream–III ebony. One of the resulting cream–III ebony males was out-crossed to an eosin female, and their eosin daughters were back-crossed to other cream–III ebony males from the general stock, which had been made up meanwhile and carried on (table 74).

TABLE 74.—P_1, *cream*-III *ebony* ♂ × *eosin* ♀; *B. C.*, w^e ♀ × *cr*III *e* ♂.

Oct. 6, 1914.	cr-III e	(w^e)	cr-III	(w^e)e	Recombination per cent.
578	74	72	64	78	49.3
579	55	58	28	24	31.5
580	107	113	21	15	14.1
581	73	55	34	45	38.1
582	87	73	11	9	11.1

As soon as this experiment began to produce a sufficient number of flies, a great variation in the amount of crossing-over in the various cultures was noticed. The ebony stock used in this experiment was supposed from previous tests (Sturtevant, 1913) to be homozygous for a modification of the amount of crossing-over in the third-chromosome. This modifier, CIII, when in the heterozygous form, greatly reduces the amount of crossing-over, and it was this kind of result that was expected. Cultures 580 and 582 of table 74 gave a low amount of crossing-over, and in the light of subsequent experiments these are known to be cultures in which the mother was heterozygous for the cross-

* It is our custom to name mutants on the basis of the change they produce from the type of the wild fly; but since in the case of the creams the stocks homozygous for the genes are indistinguishable from simple wild stocks, the names are given from the most striking characteristic, namely, their power to dilute eosin to "cream." For convenience in the discussion of this cream, the term "cream," when referring to the character or stock, is used to indicate the double mutant form eosin cream; and in those infrequent cases in which we refer to flies homozygous for the cream gene but not for eosin the absence of the eosin character is denoted by "not-eosin cream."

ing-over variation. The other three cultures (578, 579, 581), however, gave a much higher amount of crossing-over, and for this two explanations seemed possible: either the cream–III ebony stock was not pure for cream–III as supposed, in which case this high value would be comparable with the regular values obtained in experiments free from cream–III, or the eosin stock used as P_1 (in the mating of cream–III ebony male by eosin female) itself carried cream–III (in heterozygous form, since she gave a ratio of 1:1), in which case the high cross-over value is comparable with the cross-over value obtained from mothers homozygous for cream–III. While crossing-over in females heterozygous for CIII is very much lower than in females free from CIII, yet in females homozygous for CIII it is highest of all (Muller, 1916). It was not possible to decide without testing whether the high values were due to homozygous CIII on the one hand or to homozygous not-CIII on the other.

CIII FROM EOSIN STOCK.

This question was not tested until December 1915. Meanwhile the number of stocks of primary mutants had increased to such an extent that it had become necessary to eliminate as many as possible of those stocks which seemed less

TABLE 75.—*Repetition of cream-III ebony×eosin back-cross tests.*

Jan. 8, 1916.	crIII e	(w^e)	crIII	(w^e) e	Recombination per cent.
2,773.....	102	116	17	9	10.6
2,774[1].....	132	123	27	17	14.6
2,775.....	83	81	15	11	13.7
2,776[1].....	87	104	31	11	18.0
2,777.....	68	67	46	48	41.0
2,778[2].....	120	139	26	21	15.3

[1] The two cultures 2774 and 2776 produced only half as many sons as daughters owing to the presence of a lethal (lethal-II), the mutations for which must have taken place in the eosin stock some time previously.
[2] Culture 2778 gave rise to a sex-linked mutant "roof wings," which likewise had originated in the eosin stock.

generally useful. Among those discarded was the simple stock of cream–III. However, the stock of cream–III ebony was saved for this special test.

In order to see if the experiment would give the two kinds of results observed before, several P_1 matings (cream–III ebony male × eosin female) were made, and each father was saved and remated to one of his daughters. Of 6 such tests, 5 gave the low value and 1 the high (table 75). The low values correspond to the expected result that the F_1 females should be heterozygous for CIII.

The back-crosses made up from the low cross-over cultures continued to give only low values, as shown by table 76. The occurrence of a high value among such cultures would have been an indication that one of the cream–III ebony males previously used had failed to be homozygous for CIII.

If the high value for culture 2777 were not due to homozygous not-CIII condition, then it should be due to the converse cause—homozygosity for CIII;

that is, the eosin stock which had furnished the other parent for our original experiment had itself been carrying the linkage variation CIII. In this case every cream–III ebony fly from the high value culture should be homozygous for CIII, and should give, when out-crossed to eosin flies free from CIII, all

TABLE 76.—*Continuation of B. C. matings from low-value cultures (2773).*

Feb. 1, 1916.	crIII e	(w^e)	crIII	(w^e) e	Recombination per cent.
3,124.....	127	112	8	19	10.1
3,402.....	71	113	20	17	16.7
3,403.....	73	116	22	12	15.2
3,404.....	38	53	9	8	15.7
3,742.....	69	115	28	13	13.8
3,743.....	39	63	12	6	14.0
Total.....	417	581	99	75	14.8

offspring heterozygous for CIII and all daughters giving the low value. One such test was made, and the daughters all showed the low value (percentages of recombination = 10.2, 8.3, 9.0, table 77).

Both of these tests (tables 75 and 77) pointed to the presence of CIII in the particular eosin stock used in the out-cross, though they were not conclusive

TABLE 77.—P_1, *cream*-III *ebony* ♀ × *eosin Dichæte* ♂; *B. C.*, F_1 (w^e) *D* ♀ × *cr*III *e* ♂.

March 23, 1916.	crIII e (w^e)D		crIII D (w^e)e		crIII (w^e)D e		crIII D e (w^e)	
3,902.....	136	146	7	6	9	10	..	1
3,903.....	93	82	2	9	2	3	..	1
3,904.....	86	96	1	3	10	4	..	..
Total....	315	324	10	18	21	17	..	2

because of having been done on so small a scale. Conclusive proof was soon furnished by Sturtevant, who had been trying for some time to get, by crossing-over, a stock that is CIII but not ebony. He made tests of eosin stock and found some individuals that carried CIII, from which he secured the desired stock (see p. 91).

PRESENCE OF CIII IN THE CREAM-III EBONY STOCK.

The experiments of Bridges had showed that CIII was present in the eosin cream–III ebony stock. Later Muller carried out some crosses with this stock, and obtained results that indicated that no CIII was present. To test the question further, Bridges crossed crIII e to (sepia) Minute, and tested several daughters by back-crossing to cream–III ebony males (1920). Among the offspring there were no recombinations for ebony and Minute and only a few for cream–III and the other two characters. That is, in the particular fly used for the P_1, CIII was still present.

LINKAGE RELATIONS OF CREAM-III TO DICHÆTE.

In making the tests of the last experiment (table 76), the out-crosses of cream–III were made to eosin Dichæte, in order to establish by a three-point linkage experiment the location of cream–III in the third-chromosome. The preceding experiments (tables 74, 75 and 76) had given 404 recombinations in a total of 2,887 flies, or 14 per cent, in cases where the mothers were heterozygous for CIII. In other experiments (by Sturtevant and by Muller) in which ebony has been run with various third-chromosome genes in heterozygous CIII condition, the amount of recombinations for sepia and ebony has been 25, for pink and ebony 2±, and of ebony with rough 0.0+. That is, there is some crossing-over in the region to the left of ebony, but almost none to the right (ebony rough 0.0+). It seemed most probable from the large recombination per cent, 14, that cream–III lies to the left (i. e., toward sepia) and at a position between sepia and pink. A comparison of the high per cent for cream–III ebony (41.0) with the known homozygous CIII per cents for sepia ebony (48±), and for pink ebony (46±) led to this same conclusion, though less definitely. The mutant Dichæte was well adapted for this test, because of its dominance, its known position in the suspected region, and from its easy classification in the same flies with cream–III, it being a wing and bristle character. The linkage relations and double cross-over classes (table 77) showed that cream is situated to the left of Dichæte. The cream–III Dichæte recombination per cent (4.2) is smaller than the sepia Dichæte recombination per cent (14.8) found in other heterozygous CIII experiments, so that cream–III is known to be between sepia and Dichæte. The amount of recombination for sepia and Dichæte when no CIII is present is 14.1, so that the probable standard position of cream–III is about 4.0 to the left of Dichæte, or at 36.5.

CREAM-III, A DISPROPORTIONATE MODIFIER.

The occurrence of the linkage variation and its testing had diverted attention from tests of the effect of the cream–III gene in the absence of eosin as a base. Accordingly, the preceding experiment was repeated, but in such a fashion that half the flies should be not-eosin. A cream–III ebony female from

TABLE 78.—P_1, *cream*-III *ebony* ♀ × *Dichæte* ♂; *B. C.*, F_1 *D* ♀ × *c*III *e* ♂.

Aug. 22, 1916.	Red.								Eosin.							
	cIII D e		cIII D e		cIII D e		cIII D + e		cIII D e		cIII D e		cIII D e		cIII D (w^e) e	
4,990.....	24	23	1	7	..	..	..	..	9	13	1	1	1	2	..	..
4,991.....	35	38	1	8	3	2	..	..	33	32	..	..	1	2	1	..
4,996.....	5	4	..	1	..	3	..	..	20	10	..	..	1	3	..	..
4,997.....	9	13	1	2	3	..	..	..	11	17	..	..	..	..	..	..
4,998.....	50	47	..	8	1	2	..	..	45	58	2	3	..	2	..	..
Total..	123	125	3	26	7	7	..	..	118	130	3	4	3	9	1	..

the high value stock was out-crossed to a simple Dichæte male. Several of the Dichæte daughters were back-crossed singly to cream–III ebony males. The result (table 78, left side) showed that the gene responsible for the dilution of eosin to the cream-color gave, by itself, a color somewhat similar to purple, that is, a "magenta." But when separations were attempted it was found that this color was not sufficiently distinct from the wild-type to make classification accurate. In the eosin half of the flies, on the other hand, the separation was easy and entirely accurate. That is, while the so-called "cream–III" gene by itself gave a certain effect, it gave so much more marked an effect in the presence of eosin that it was decided to retain the name "cream–III" instead of renaming the mutant "magenta." The separations in the not-eosin half of table correspond roughly to those of the eosin half, but the large size of the ebony not-magenta class is certainly due to the impossibility of distinguishing the "magenta" character, even though it was undoubtedly present in most of the 26 "ebony" flies of table 78.

If this character were accurately classifiable (as "magenta") without first laboriously combining eosin with all the flies used in the matings of each experiment, it would be more useful, even if less interesting from our present viewpoint. However, an experiment planned through eight generations had to be abandoned because it was found impractical to distinguish between the cream–III and the not-cream–III where eosin was not present as a sensitizer. But this abortive experiment revealed that the ebony was a disturbing factor—that the distinction was sharper in the not-ebony flies than in ebony flies. A new possibility arose, namely, that the mutation might be used (as "magenta") by excluding ebony from all the experiments. While this has not been adequately tested, it seems possible that, with experience, one may be able to use this eye-color without a preliminary eosinization of all the stocks, though it is not to be denied that in the presence of eosin the ease and speed of classification is greater.

DISPROPORTIONATE MODIFICATION.

The term "disproportionate modifier" might be used to describe the effect of cream–III upon eosin and of purple upon vermilion (see Carnegie Publication No. 278, p. 170). Most of our mutations are what may be called "general modifiers," since their effects seem to be independent of one another, and the combined effects are cumulative and roughly proportional to the degree of each single modifier. "General modifiers" may be represented by the familiar parallelogram, in which the initial condition (wild-type) is one corner, the effect of each gene acting independently is represented by the length and direction of the two adjacent sides, and the combined effect (double mutant type) is the opposite corner (fig. 13*a*). In the case of completely specific modifiers, such as cream–II, the length of the side between wild-type and (not-eosin) cream–II is zero, so that the parallelogram becomes reduced to a triangle (fig. 13*b*). In such cases as cream–III there is an intermediate condition (fig. 13*c*) in which the side between eosin and the double type is disproportionately greater than the distance between the wild-type and the simple modifier. There is another type of disproportionate modification, ex-

emplified by eosin sepia, which might be called "reversed." Sepia is considerably darker than the wild-type, becoming a purplish black in old flies. It might be expected that sepia would cause a proportional darkening of eosin, so that eosin sepia would be as much darker than eosin as sepia is darker than the wild-type. This is not the case; the eosin sepia double form is lighter than eosin, about as represented in figure 13*d*. There are several other types of disproportionate modification. Thus, for example, ruby (sex-linked) may be described as a "non-modifier" of pink. The effects of these two genes are in the same direction and of like amount; but they fail to have a cumulative effect, the double form being practically indistinguishable from either single

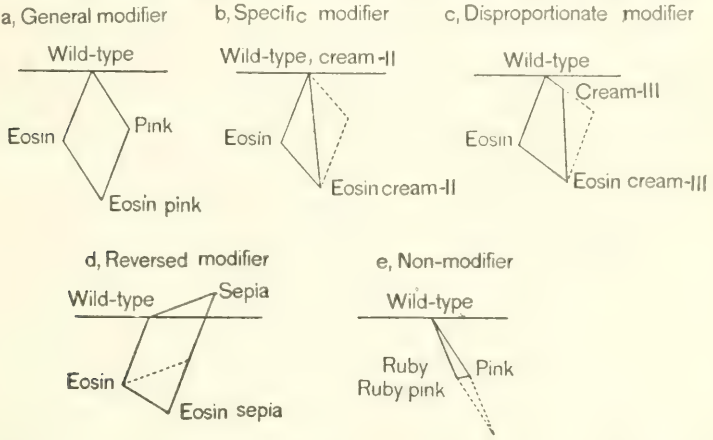


FIG. 13.—Types of modification. (*a*) proportionate modification. (*b*) specific modification. (*c*) disproportionate modification. (*e*) reversed modifier. (*f*) non-modifier.

type, instead of being as much lighter as each is lighter than the wild type (fig. 13*e*).

DESCRIPTION OF CREAM-III.

A pure stock of the sex-linked eye-color eosin shows a marked secondary sexual difference. The eye-color of the eosin female, as seen in pure-breeding cultures, is a rather deep pink, only slightly yellowish, while the eye-color of the eosin male is a pinkish yellow, much lighter in tone than the color of the female. All double-mutant forms involving eosin, for example, eosin vermilion and eosin pink, are likewise bicolored (Morgan and Bridges, 1913). This difference is seen in the cream–III stock, where the color of the male is paler than that of the female and less pink in tone. The male is cream or straw or yellow-ivory in color.

In figure 14 is given a graphic representation of the difference in grade between cream–III males and females, as well as a comparison with the other creams and with eosin.

The color of the not-eosin cream–III is a maroon or garnet, like the color of the mutant purple (see Carnegie Publication No. 278, p. 170), but departs less widely from the red of the wild-type.

THE MODIFICATION OF CREAM-III BY EBONY.

The presence of ebony makes the eye-color of cream–III slightly paler and pinker (less yellow) as compared with not-ebony creams (compare figs. 5 and 8, plate 1). In not-eosin cream–III the presence of ebony decreases the ease of separation (compare figs. 4 and 7, plate 1). This effect upon not-eosin cream–III is apparently in the reverse direction from the effect upon eosin

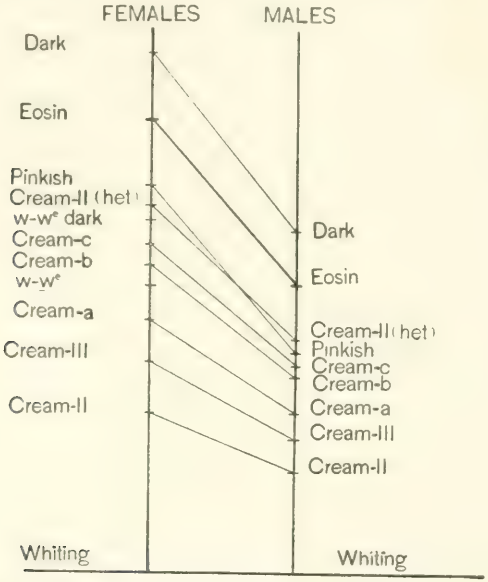


FIG. 14.—Grades of cream-III, male and female, compared with other creams and with white.

cream–III; in the first case the modification is less of a departure from the wild-type, and in the second a greater.

EVALUATION OF CREAM-III.

Aside from its value in studying the effects of interaction of genes, cream–III has little usefulness. The difference between cream–III and wild-type in the absence of eosin is so slight that separations would in any case be slow. The locus (4 units to the left of Dichæte) is a valuable one and this region is not covered by any other mutant.

GIANT (gt).

(Plate 3, Figure 4.)

ORIGIN OF GIANT.

In order to determine whether the gene for the recently discovered mutant spineless was in the third chromosome, Bridges crossed spineless to the known

third-chromosome recessive sepia, and raised an F_2 culture (table 79). The F_2 spineless flies were slow to hatch, and when they appeared they were seen to be much larger than normal (September 28, 1914). Careful watch was kept of the culture, and it was noted that the "giant" flies continued to appear, and were, in fact, cleanly separable from the normal flies. None of

TABLE 79.—P_1, *spineless giant white-ocelli* ♀×*sepia* ♂: F_2, F_1+♀×F_1+♂.

Sept. 24, 1914	+	se	ss gt wo	gt wo	ss wo
564.....	77	34	23	2	5

the giants were sepia. Most of the giants were spineless but 2 were not, and there were 5 spineless flies that were not giants.

CHROMOSOME AND PROVISIONAL LOCUS OF GIANT.

The fact that none of the giants were sepia showed that the gene for giant is in the third-chromosome. The mutation had occurred in the spineless stock. The spineless not-giants and the not-spineless giants that occurred in F_2 were cross-overs. On the basis of these data, the locus of giant was supposed to be about 25 units from spineless, in the third chromosome.

The occurrence of the giant mutation in a stock that already contained the two third-chromosome recessives spineless and white-ocelli, furnished a triple recessive needed in order to locate the gene for giant by means of a single three-point back-cross test. F_1 wild-type females were obtained by crossing spineless giant white-ocelli females to wild males (table 80, culture 767).

TABLE 80.—*Spineless giant white-ocelli females by wild males.*

Nov. 17, 1914.	+♀	+♂
767.....	54	52
1,033.....	67	73
1,223.....	18	17
1,241.....	52	61
Total.....	191	203

At the same time that this P_1 mating was started, spineless giant white-ocelli males and females were mated together to provide a stock of the triple recessive for use in back-crossing. Two such mass-cultures for stock were started, but both failed to produce offspring. However, 3 spineless giant white-ocelli females had been mated to 2 of their spineless not-giant brothers, and this culture produced 106 spineless white-ocelli and 88 spineless giant white-ocelli flies (culture 688, October 30, 1914).

MALE-STERILITY OF GIANT.

Six back-cross cultures were started by mating the F_1 wild-type females to the spineless giant white-ocelli males. All of these pair cultures failed to produce offspring, although the flies were transferred to fresh culture-bottles and given a second trial. Also, three more mass-cultures of giant by giant, for stock, were unproductive. The flies of these later cultures were then separated and the females were mated to not-giant brothers and the males to not giant sisters. All of the male giants were completely sterile, although most of the females were fertile. The output of the female giants was not large, and subsequent work showed that the productivity of giant females is very low. About 30 giant males were tested at this time, and all proved sterile. Many more were tested later with like result.

This case offered a remarkable inverse of the case of dwarf, that had been found only a few weeks previously (see p. 101), in which the males are completely fertile and productive, while the females are completely sterile. The case of morula (see Carnegie Publication No. 278, p. 230) was another of unisexual sterility in which the female was sterile. Later, several such cases of female-sterility, and another of male-sterility, viz, cleft, were found; and these mutants, together with rudimentary, were given to Miss Clara J. Lynch, who made extensive tests of the sterility in the different cases (see Lynch, 1919).

THE SPINELESS GIANT WHITE-OCELLI F_2 RESULTS.

Because of the sterility of giant males it was not possible to carry out female back-cross tests of the usual type. Accordingly two F_2 cultures were raised for a preliminary test of the position of the giant gene with reference to those of spineless and white-ocelli (table 81). The ratios among the flies

TABLE 81.—*P_1, spineless giant white-ocelli ♀ × wild ♂: F_2, F_1 + ♀ × F_1 + ♂.*

Dec. 2, 1914.	+	ss	wo	ss wo	ss gt wo	gt wo	ss gt	gt
879....	128	6	16	11	32	4	6	0
1,042 ...	140	3	11	14	17	0	4	0
Total..	268	9	27	25	49	4	10	0

that were giant were equivalent to those in back-cross results. The first point to be noted is that the locus of giant is between that of spineless and that of white-ocelli. This is proved by the absence of representatives of the (double-recombination) class not-spineless giant not-white-ocelli. For spineless and giant there were 4 recombinations (giant white-ocelli), and for giant and white-ocelli 10 recombinations (spineless). The first F_2 culture (table 79) gave 2 recombinations for spineless and giant in a total of 25 giant flies. There was thus a total of 6 recombinations for spineless and giant in 88 giant flies, which gives a recombination per cent of 6.8. The 10 recombinations for giant and white-ocelli in a total of 63 gives a recombination per cent of 15.8. There

is normally 17.7 per cent of recombination for spineless and white-ocelli. The calculated position for giant is at 64, or 5.5 units to the right of spineless. On account of the small number of flies, this position is not very well determined.

THE DIGENIC NATURE OF GIANT.

Among the F_2 flies from the cross of spineless giant white-ocelli to wild there were 25 that were spineless not-giant white-ocelli. These could not be due to double crossing-over, since their number was about half that of the original combination class, and there is known to be less than 1 per cent of double-recombination for the spineless white-ocelli section. Neither is it possible that these were really giant flies that could not be distinguished from the not-giant type; while there is some fluctuation in the giant character, it is not enough to account for more than a very few such flies at most. The most probable explanation appears to be that the expression of the giant character is dependent upon two mutant genes, one in the third chromosome about 6 units to the right of spineless, and another in some other chromosome, probably the second.

More light was thrown on this situation by the results of some male back-cross tests, as follows: A spineless giant white-ocelli female was crossed to a wild male, and F_1 wild-type males were crossed individually to spineless giant white-ocelli females. Most of these cultures failed, and for this reason the cross was repeated for several different generations, with the rather meager results of table 82. Great care was taken in the classification of the giant

TABLE 82.—*P_1, spineless giant white-ocelli ♀ × wild ♂; B. C., F_1 + ♂ × ss gt wo ♀.*

Oct. 6, 1915.	+	ss gt wo	ss wo
2,215	54	17	6
4,925	6	4	1
4,965	65	24	11
4,969	30	9	12
4,972	56	17	9
4,973	44	15	5
5,146	22	10	0
5,167	23	10	4
5,356	51	14	11
Total...	351	120	59

character, and it was found to be a fact that spineless not-giant white-ocelli flies were occurring in the male back-cross. This showed that the giant character is really due to two genes, and that the locus of the second gene is not in the third chromosome. The ratio of 25 ss wo to 49 ss gt wo, observed in the F_2 results of table 81, could have been explained as due to a second gene in the third chromosome which gives about 34 per cent recombination. But in that case none of the spineless white-ocelli flies from the male back-cross could have failed to be giant, since there would have been no crossing-over to remove the second gene.

THE SECOND GENE A DOMINANT THAT IS LETHAL WHEN HOMOZYGOUS.

It was noted that the ss wo class of table 81 was about half as large as the ss gt wo class (25:49). This same 1:2 ratio was again observed for these classes as they appeared in the results of the male back-crosses of table 82 (totals 59:120). Evidently two-thirds of the offspring receive the second gene, while one-third fail to receive it. Such 1:2 ratios are a familiar product in *Drosophila*, and indicate that a dominant that is lethal when homozygous is present in both parents in the cross. Dichæte may be used as a type of such genes. The character Dichæte is always the result of the presence of a dominant gene in one of the two third-chromosomes. Out-crosses of Dichæte give 1:1 ratios of Dichæte to not-Dichæte, in keeping with the heterozygous nature of Dichæte. When two Dichæte flies are mated together, the result is two Dichæte: one not-Dichæte. One class, the homozygous Dichæte, is missing from the typical 3:1 ratio. That it really is the complete absence of this homozygous class that is responsible for the 2:1 ratio has been proven by the simultaneous use of other mutant characters, which are known not to be lethal but which can be made to appear in corresponding aberrant ratios according to the way that they are combined with Dichæte in the cross.

Just as two-thirds of the flies that are spineless and white-ocelli receive also the dominant second gene, so also among the wild-type flies of the male back-crosses of table 82 there should be about one-third of the flies that do not carry the second gene. Accordingly, one-third of the back-cross cultures should be from males free from the dominant gene. These should produce 1:1 instead of 2:1 ratios of ss gt wo: ss wo. Examination of the nine cultures of table 82 show two (4969 and 5356) that are probably such 1:1 ratios.

There is an even more decisive test that can be applied: All of the ss gt wo flies should carry the second gene, and all of the ss wo flies should be free from it. If such flies are mated together the result should be 1:1 ratios. One such cross was made in the early work on giant. Three ss gt wo females from an F_2 culture were crossed to ss wo brothers, primarily for the purpose of providing a stock of the triple recessive. This culture (No. 688, referred to already on p. 121) produced 88 giant to 108 not-giant, which is a fairly satisfactory 1:1 ratio, considering the mortality of giants and the probable error for this number of flies.

One other test is available. One-third of the F_2 cultures from the cross of spineless giant white-ocelli to wild should fail to produce any giants whatever, although the grandmother was giant. Two such cultures were found (1369 and 1370), but unfortunately the counts were discontinued when it became apparent that no giants were present. Both spineless and white-ocelli appeared in these cultures in the normal relations.

An attempt was made, by use of the second-chromosome dominant Star, to prove definitely that the dominant second gene is in the second chromosome, and also to determine its locus within the chromosome. None of the many cultures produced a decisive result, and before the attempt could be repeated, the stock of giant was lost by reason of its infertility and low productivity.

GIANT A CASE OF RECIPROCAL SPECIFIC MODIFICATION.

Giant flies are, then, the product of a recessive gene situated about 6 units to the right of spineless in the third chromosome, and of a dominant gene that is lethal when homozygous and that is situated in another autosome, probably the second. Since the spineless white-ocelli flies that occurred in the crosses, and also the other not-giant flies (two-thirds of which are heterozygous for the dominant second gene) showed no effect of these genes, we must suppose that each of these genes is a specific modifier of the other. The case is thus similar to that of vortex, worked out by Bridges and Mohr (Genetics, 1919, p. 283), and of Ski (see p. 149).

PORT.

In March 1914, Morgan found an eye-color mutant present in considerable numbers in one of his stock cultures. The color of the eye was similar to maroon, but was an even slighter departure from the wild-type. The character was called "port," from the similarity of the color to that of port wine. The separation of "port" from the wild-type was slow and difficult, although

TABLE 83.—*P_1, port ♂ × vermilion ♀; F_2, F_1 + ♀ × F_1 v ♂.*

Sept. 24, 1914.	+	v	port	v port
568.....	57	59	24	21
569.....	36	55	17	23
Total....	93	114	41	44

probably fairly complete. A pure-breeding stock of "port" was obtained by Morgan and was turned over to Bridges, who found from an F_2 of "port" by ebony that the gene for "port" is in the third chromosome.

Because of the similarity between "port" and the third-chromosome mutants maroon and pink, "port" was crossed to maroon and pink as a test for possible allelomorphism. Both of these crosses gave only wild-type offspring (port × maroon, culture 502, 55 wild-type; port × pink, culture 501, 34 wild-type), from which it is apparent that port is allelomorphic neither to maroon nor to pink.

A "port" male was out-crossed to a vermilion female and the two F_2 cultures were raised (table 83). In the first culture 28 per cent of the flies were "port," and in the second 33 per cent, both somewhat too high on the basis of the expected 25 per cent.

On account of the slight difference between "port" and the wild-type, it was not thought worth while to carry out any further tests at that time, and the stock was turned into the stock-room. About 2 years later it was noticed that the stock supposed to be "port" was practically indistinguishable from a stock of wild flies. It was decided that the stock had become contaminated or that an inhibitor had arisen. Rough tests were made by out-crossing males from the "port" stock to eosin and to vermilion females and raising F_2 pair-

cultures (tables 84 and 85). The results indicated that the "port" stock contained flies of different constitution; the F_2 cultures from one male gave no "port" at all (Nos. 5524, 5525); the F_2 cultures from two other males gave about a sixth of the flies "port" (Nos. 5522, 5523, 5553); while the F_2 cultures from the remaining male contained about one-third "port" flies (Nos. 5548, 5549).

TABLE 84.—P_1, *port?* ♂ × *vermilion* ♀; F_2, F_1 + ♀ × F_1 *v* ♂.

Oct. 14, 1916.	+	v	port	v port
5,524.....	109	104	0	0
5,525.....	85	58	0	0
5,553.....	75	76	14	18

The outstanding fact in connection with these crosses is that flies from a stock that itself showed only normal eye-color gave in F_2 large proportions of flies with a mutant "port." This fact proved that the stock had really contained the gene for "port" but that there was present in addition some sort of inhibitor. The cultures in which "port" appeared as approximately a sixth of the flies are easily explained on the ground that the grandfather had in those cases been homozygous for "port" and also for an inhibitor that is

TABLE 85.—P_1, *port?* ♂ × *eosin* ♀; F_2, F_1 + ♀ × F_1 w^e ♂.

Oct. 14, 1916.	+	w^e	port	w^e port
5,522.....	98	97	20	18
5,523.....	103	64	18	20
5,548.....	106	102	39	29
5,549.....	81	76	28	43

dominant and not lethal when homozygous. Three-sixteenths or 18.8 per cent of the flies should be "port," which is closely approximated by the 17.4 per cent actually observed. It is not clear what the explanation of the other cases is, but it seems that they are even more complex, requiring the assumption of a lethal or some other factor, such as contamination or another inhibitor. These cases were not followed further, and the stock was lost soon afterwards, so that the test can not be repeated.

SAFRANIN.

ORIGIN OF SAFRANIN.

In testing the linkage relations of the second-chromosome recessive plexus, Bridges found a maroon-like eye-color which was traced back to the black plexus stock (January 15, 1915, culture 1227). These "safranin" flies were inbred and produced a pure-breeding stock.

CHROMOSOME OF SAFRANIN.

A black plexus safranin male was out-crossed to a female of the third-chromosome recessive spineless, and an F_2 culture was raised from a pair of the wild-type F_1 flies (culture 1431, February 24, 1916; +138, saf 60, ss 59, saf ss 0). In F_2 none of the spineless flies were safranin, while the safranin character was distributed apparently at random among the black and plexus flies, from which facts it follows that the locus of safranin is in the third chromosome.

Safranin was crossed to maroon and to pink, and only wild-type flies were produced in F_1.

Safranin females were out-crossed individually to wild males, and F_2 and F_3 cultures were raised to free the stock from black plexus. A stock was obtained that seemed to be free from these second-chromosome mutants, and this was turned in to the stock-room. Shortly afterwards this stock was lost, so that nothing further is known of the genetic behavior of safranin.

Later a similar eye-color was found in a black stock, and the gene of this new safranin (safranin-b) was found to be in the second chromosome.

DICHÆTE (D).

(Figure 15.)

ORIGIN OF DICHÆTE.

A sex-linked mutation called "cleft" venation had been found, and the linkage experiments showed that its locus occupied a new extreme right-end

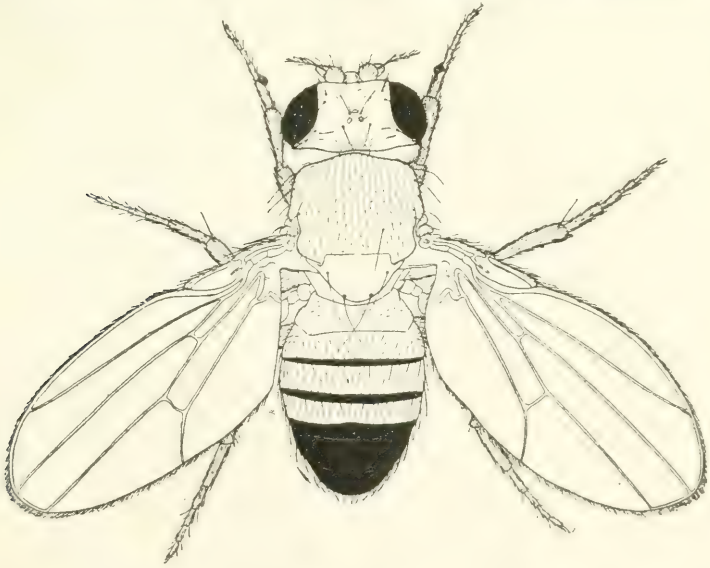


FIG. 15.—Dichæte wing and bristles.

position in the X chromosome. The locus of cleft is at 5.5 units beyond fused, which was the right-most locus previously known. Cleft males are absolutely sterile, and in consequence no cleft female has ever been produced. Moreover,

no back-cross experiments to determine the locus of cleft can be carried out. The position of cleft was found, however, by mating females heterozygous for cleft to males having the two well-established characters sable (locus 43.0) and forked (locus 56.5). F_1 females were tested by sable forked males. The sons of those F_1 females heterozygous for cleft gave three-point linkage data.

Among the offspring of one such pair-mating, Bridges found a single female whose wings were extended at a wide angle and elevated (culture 1817, July 3, 1915). Besides the divergent wing character there was present also a bristle character. Only the two posterior dorso-central bristles remained, the two anterior bristles being entirely absent. These features were so sharply defined that it seemed probable that they were the result of mutation.

DOMINANCE OF DICHÆTE.

Since only a single individual showing the mutant character had occurred among the many offspring of the pair-mating, it seemed probable that the mutant character was not a recessive. The appearance of a recessive requires that both parents be heterozygous for it, in which case a quarter of the offspring show the recessive character. A sex-linked recessive could appear as a single individual if the mutation had occurred in the germ-cells of the mother. Such an individual would be a male. The "Dichæte" individual was a female. This indicated a dominant mutation.

CHROMOSOME OF DICHÆTE.

The wings of the Dichæte female were so similar to those of "bent," a fourth-chromosome character, that the first test made was to cross Dichæte to the other fourth-chromosome recessive "eyeless." Half of the F_1 flies were

TABLE 86.—P_1, *Dichæte* ♀ × *eyeless* ♂.

July 15, 1915.	+♀	+♂	D♀	D♂
1,889.....	30	18	23	24
B. C., F_1 Dichaete ♂ × eyeless ♀.				
July 29, 1915.	D	ey	D ey	+
1,969.....	67	46	47	36
1,970.....	43	47	31	42
Total.....	110	93	78	78
F_2, F_1 Dichaete ♀ × F_1 Dichaete ♂ (eyeless unclassified).				
Aug. 2, 1915.	Dichaete.		Not-dichaete.	
2,027.....	277		105	

Dichæte, which confirmed the view that the character was a dominant (table 86), for which the mother had been heterozygous. Some F_1 Dichæte males were then back-crossed to eyeless females. All four possible classes appeared in the offspring, which proved that the gene for Dichæte is not in the fourth chromosome. The back-cross results also furnish data upon the independence of the Dichæte-bearing chromosome and the fourth chromosome; 43.5 per cent of the flies were recombinations, where 50 per cent was expected. This deviation, which is the largest met with in such crosses, is about $2.5 \times \sqrt{Npq}$, but under the circumstances is not regarded as significant.

Similar back-crosses were started to test the relation of Dichæte to the second and to the third chromosome. A Dichæte male was crossed to a black vestigial female (culture 2028, + 162, D 166) and the F_1 Dichæte males were back-crossed to black vestigial females. The back-cross counts were discontinued, since male back-cross tests of Dichæte by peach sooty began to hatch at the same time, and these proved conclusively that the locus of Dichæte is in the third chromosome (F_1 culture, August 3, 1915, + 295, D 271; for male back-crosses, see table 87).

DICHÆTE × PEACH SOOTY.

From the same F_1 mating (Dichæte × peach sooty) that had furished proof that the locus of Dichæte was in the third chromosome, female back-cross tests were made by mating the F_1 Dichæte females to peach sooty males (table 87). The results constituted a three-point back-cross in which two of the loci (peach and sooty) were already accurately mapped. In form the experiment was perfectly adapted to determine the locus of the one unknown, Dichæte. In the back-cross cultures the smallest pair of parallel classes were Dichæte

TABLE 87.—*P_1, Dichæte ♀ × peach sooty ♂; B. C., F_1 D ♂ × p^p e^s ♀.*

Aug. 15, 1915.	D	p^p e^s
2,067	167	167
2,068	135	123
2,069	103	116
Total	405	406

B. C., F_1 D ♀ × p^p e^s ♂.

Aug. 15, 1915.	D	p^p e^s	D p^p e^s	+	D e^s	p^p	D p^p	e^s
2,070	136	158	3	2	34	24	0	3
2,071	112	110	4	7	24	23	1	3
2,072	141	149	5	6	40	30	1	1
2,073	125	134	5	6	39	28	2	2
2,074	86	96	3	2	21	19	1	3
2,075	179	193	8	8	41	48	6	4
2,140	45	38	0	0	15	15	0	2
Total	824	878	28	31	214	187	11	18

peach and sooty, which together constituted 29 flies out of the total of 3,111. Where the numbers are large enough to be statistically significant, as in this case, the smallest classes are invariably due to double crossing-over, and this fact enables us to determine unambiguously the chromosomal order of the loci. The order of peach and sooty in the chromosome was known, sooty being to the right of peach. The only position relative to these in which Dichæte could be situated and give the observed double-cross-over classes is to the left of both peach and sooty. This position is in complete agreement with the recombination per cents as calculated from the observed numbers. There were 460 recombinations for peach and sooty, which is about the usual value. There were 88 recombinations for Dichæte and peach, which means 4.1 per cent of recombination. In order that the locus of Dichæte should conform to the indicated position to the left of peach, the Dichæte peach value should be greater than the peach sooty value, as it is (21.3 > 19.8).

DICHÆTE × "PEPLE"

While the above back-cross was in the F_1 stage, a slightly more complicated back-cross was started, in which Dichæte was mated to the multiple recessive

TABLE 88.—*Dichæte ♀ × "peple" ♂; B. C. F_1, D ♀ × "peple" ♂.*

Sept. 22–Oct.	D p^p ss e^+ ro	D p^p ss e^+ ro	—	D ss p^+ ro	D p^p ss e^+ ro	D p^p ss e^+ ro	D p^p s^s e^+ ro	D p^p ss e^+ ro	D p^p ss e^+ ro	D p^p ss e^+ ro	D p^p ss e^+ ro
2 188	78 82	6 3		8 11	14 12	17 17	0 2	0 1	1 0	1 0	0 3	1 1
2 189	90 93	2 5		12 9	11 11	18 20	3 3	0 0	3 2	1 0	4 0	0 0
2 406	61 46	1 6		2 8	3 3	6 11	0 0	0 1	1 0	1 1	0 0	0 0
Total	229 221	9 14		22 28	30 26	41 52	3 5	0 2	5 2	3 1	4 3	1 1

"peple" (peach spineless sooty rough). The results of the back-crosses (table 88) fully confirmed the simpler experiment. The Dichæte peach recombination per cent of 5.4 is nearer the standard per cent, 7.3.

DESCRIPTION OF DICHÆTE.

The most striking feature of Dichæte flies, and the one mainly used in the separations, is the position of the wings, which are held out at an angle of about 45° from the sides of the body, or at right angles to each other. This position is the resultant of two bends in the wing. The bases of the wings extend out at right angles to the body and are considerably thickened. Just beyond the base there is a second bend in the wing, so that the main part of the wing is turned backward by about 45° from its initial direction. The wings are elevated as well as held out from the body. Each wing is held up about 30° above the horizontal. There is a very high degree of constancy in the position of the wings. In normal flies that are over-etherized the wings are held upright above the back; this is true also of over-etherized Dichæte

flies; but the two kinds can be separated easily because of the narrowed and extended base of the Dichaete wings. The texture of the Dichaete wing is entirely normal, which is in contrast to most of the other mutants having extended wings. For example, in vestal, in which the wing position is much the same as in Dichaete, the wing is very thin and delicate and is paler than normal in color. Because of their elevated posture and normal texture, the Dichaete wings seldom become bedraggled. The venation is clearly visible, and the Dichaete character can thus be used with a large range of other wing characters without masking effects.

In case the Dichaete fly has just emerged from the pupa-case and the wings are not yet expanded, or in case of accident to the wings, the classification is made as certainly, though not as rapidly, by means of the bristles. The first Dichaete fly seen had only 2 instead of 4 dorso-central bristles. The 2 anterior ones were missing. In subsequent cultures it was seen that there was variation in the number of the dorso-centrals, and also, in more extreme types, of the scutellars. Stuttevant made an extensive study of the distribution of the Dichaete bristles (Carnegie Publication No. 284, 1919) and found that normally no Dichaete flies occurred in which all four dorso-centrals were present. Only rarely were as many as three present, and in this case the single anterior one was undersized. The modal case seems to be the presence of a single posterior dorso-central and of all four scutellars (so-called 2).

Somewhat less frequent were the two approximately equal classes in which one or two (posterior) dorso-centrals were present. Rarely all the dorso-centrals and one of the scutellars were missing. If special methods are present this range is extended in a + or a − direction so that strains were obtained that threw an occasional fly with all the dorso-centrals present, and other strains in which an occasional individual lacked all of the dorso-centrals and all but 2 or 1 of the scutellars. Of these 4 bristles, the anterior dorso-centrals were the most sensitive and the posterior scutellars the most refractory to elimination. There is a slight but significant sexual dimorphism in the Dichaete flies, the males averaging about a tenth of a bristle lower than the females. A bristle character that was long overlooked, though it is probably the most invariable of the Dichaete characteristics, is the absence of the post-alar bristles. Normally the anterior post-alars are very large bristles, though they are not conspicuous because of their situation on the side of thorax near the wing. The posterior post-alar are normally rather small bristles located nearer the posterior dorso-centrals. Both of these bristles are regularly absent from Dichaete flies; occasionally the posterior ones are present, and more rarely the anterior. But these bristles are never of normal size when present.

When bristles are absent from Dichaete flies, the absence includes the thickened ring in which the bristle is mounted. This ring remains in the case of bristles that are absent from Hairless flies.

The only other effect of the Dichaete gene known is an occasional deformity or infolding of the back of the head, with an extension of the "crown" of hairs to a greater area on the back of the head.

The Dichaetes are typically large, vigorous flies, with head abdomen, but whether these size differences are significant is not determined.

THE LETHAL NATURE OF HOMOZYGOUS DICHÆTE.

The first cross of Dichæte to Dichæte (culture 2027, table 86) produced 277 Dichæte to 105 not-Dichæte. From this culture several others were raised in the next generation in order to secure a stock free from the fourth-chromosome recessive eyeless. In these the ratio of Dichæte to not-Dichæte was approximately 2:1, rather than 3:1 as suggested by the first culture. Moreover, none of the cultures showed any evidence of breeding true to Dichæte. These facts were taken to mean that Dichæte is lethal when homozygous, as was known to be the case with Star and two or three other dominants.

A more conclusive test was carried out by using the recessive pink in combination with Dichæte. Pink is known to show normal viability, yet when a fly that was both Dichæte and pink was out-crossed to wild and an F_2 culture raised from a pair of the F_1 Dichæte flies, only two flies were pink in a total of 117 (culture 3432; D=77, +=38, D p=2, p=0). There is normally about 6 per cent of recombination for Dichæte and pink, and these two Dichæte pink flies are such recombinations. On the assumptions that homozygous Dichæte is lethal and that there is no crossing-over in the male, the F_2 zygotes should be in the ratio D=2*oc*+1*rc*: +=1*oc*+1*rc*: D p=1*oc*: p=0. (Each original combination class of eggs is designated *oc* and each recombination class *rc;* the gametic series in the eggs is thus: D p=*oc*, +=*oc*, D=*rc*, p=*rc*. With a Dichæte pink recombination per cent of 6, *rc*=3 and *oc*=47). The amount of recombination that actually occurred in the above cross can be calculated from the Dichæte pink class (*rc*=2) and the wild-type class (*oc*+*rc*=38) to have been 5.3.

Subsequent work with Dichæte has never given results that suggest that homozygous Dichæte lives. Many experiments have been such that they gave incidental evidence on the lethal nature of homozygous Dichæte.

EXTENDED, AN ALLELOMORPH OF DICHÆTE.

One allelomorph of Dichæte viz, Extended, is known (see p. 165 for a full account of Extended). This is a dominant, but overlaps the wild-type to such an extent that it is not useful. It is lethal when homozygous, and the Dichæte-Extended compound is also lethal. There are no recorded reoccurrences of the Dichæte mutation.

AN ANALYSIS OF THE EFFECTS OF SELECTION OF DICHÆTE BRISTLES.

In connection with the description of the Dichæte character, mention has already been made of the variation in the number of bristles of the Dichæte flies, and of the selection experiments carried out by Sturtevant. These experiments and findings (for a full account see Carnegie Publication No. 264) may be briefly summarized as follows: "Plus" and "minus" lines, differing on the average by about 1.5 bristles, were established by selection. It was found that continued close inbreeding decreased the effectiveness of later selection, and apparently also decreased the variability in bristle-number. When plus and minus lines were crossed, F_1 was intermediate and F_2 showed a greatly increased variability. Linkage tests demonstrated that there were modifiers for bristle-number present—at least one in the second chromosome and one in the third (see p. 132). These modifiers are sufficient to account for the observed results of selection and crossing.

EVALUATION OF DICHÆTE.

Dichæte is the most valuable third-chromosome mutant thus far known. It is invariably dominant. There is very little fluctuation in the degree of development of the wing character which is the feature most used in the separations. Bristle characters are present, which are subject to variation but which never overlap the wild-type. The separations are complete, and in point of ease and speed of classification Dichæte is unsurpassed. When homozygous, Dichæte is invariably lethal; when heterozygous, Dichæte is not inferior to the wild-type in viability. The fertility and productivity of the mutant are normal.

The locus of Dichæte is in the third-chromosome about 7.6 units to the left of that of pink, which is the primary base of reference of Dichæte. Dichæte is about 14.4 units to the right of sepia, which is the secondary base of Dichæte and which was for long the zero of the third chromosome. The new zero, or left-most known mutant, is roughoid. Dichæte is about 40.4 map-units to the right of roughoid.

The locus of Dichæte lies in the left limb of the third-chromosome, not far to the left of the mid-point and in a region that is peculiarly susceptible to variation in crossing-over. Thus, Dichæte is valuable in studying the influence of age, of temperature, and of genetic factors upon crossing-over and coincidence. The volume of linkage data involving Dichæte is very large, as can be seen from the summary of table 2. The importance of Dichæte is so much greater than that of any other third-chromosome mutant that it is now used as the real base of reference of the third-chromosome. In this regard it is comparable to black in the second chromosome.

Dichæte has been used in testing the chromosome group of most mutants that have been found since the discovery of Dichæte. When a new mutant is found that is not supposed to be sex-linked, it is immediately mated to a Star Dichæte fly from a special stock that is maintained for this purpose. Star is a second-chromosome dominant and Dichæte is a third-chromosome dominant. From such matings F_1 Star Dichæte males heterozygous for the mutant are back-crossed to females homozygous for the mutant, if such are at hand. If no mutant-type females are at hand, the F_1 Star Dichæte males are mated to their not-Star not-Dichæte sisters which are heterozygous for the mutant gene. The F_2 flies that show the mutant character are then the equivalent of the products of a male-test back-cross Because there is no crossing-over in the male these mutant-type F_2 flies, in case the gene for the mutant is in the second-chromosome, will all fail to be Star, but will be equally Dichæte and not-Dichæte. But if the mutant is in the third chromosome, then conversely, none of the mutant-type flies will be Dichæte, while half will be Star and half not-Star. By means of this single cross it can be determined to which of the four chromosomes the mutant belongs. The distribution peculiar to sex-linkage will show whether or not the mutant is in the first chromosome. If then a mutant is not sex-linked and shows linkage neither to Star nor to Dichæte, the gene is known to be in the fourth chromosome. The procedure in determining the chromosome of dominant mutants is the same, except that F_1 Star Dichæte mutant-type males are out-crossed to wild females. An earlier method of determining the linkage group to

which a new mutant belongs was to mate the mutant to a fly carring a second-chromosome and a third-chromosome recessive, usually black and pink. F_1 wild-type males and females were mated together, and the diagnosis was made on the basis of the 9:3:3:1 and 2:1:1:0 ratios that resulted. Thus, the presence of a black mutant-type class is correlated with the absence of a pink mutant-type class and means that the gene for the mutant is a member of the third group. With the older method, the next step was to synthesize the double recessive pink mutant-type, and this often proved very laborious. With the present method, a Dichæte mutant back-cross can be made by simply mating together the Dichæte females and mutant-type males from the cultures that show that the given gene is in the third-chromosome. Another and serious difficulty with the old method is the presence of the other recessive. Thus, if the double recessive pink mutant-type is made from the F_2 of the cross to black pink, black will be present as an impurity in the stock secured, and will prevent the use of the stock with ebony. The black can be eliminated by selection, which at best is a time-consuming operation. To avoid this complication, the first step taken with a new mutant was usually not the determination of its chromosome, but was the establishment of a stock of the mutant from which flies were used in making the black pink F_2 test, and other flies were used in starting the synthesis of the needed multiple recessives. All these precautions meant delay and labor; with the Star Dichæte method the Star is a dominant and can be thrown out at pleasure with no loss of time or uncertainty of result. The Star Dichæte stock is used more frequently in experiments than any other of the 250 or more stocks that we maintain.

Just as Dichæte is the first mutant to be used in determining the chromosome of a new mutant, it is generally the first to be used in determining the locus of the gene within the chromosome. A Dichæte mutant back-cross can be immediately started from the culture that showed that the mutant is in the third chromosome. This preliminary experiment gives sufficient indication of the locus of the mutant for more definite experiments to be planned. But there is a better method than to make a simple Dichæte back-cross. There is another third-chromosome dominant that is second only to Dichæte in usefulness, viz., Hairless, whose locus is in the right limb of the third-chromosome and some 29 units from Dichæte. As soon as it is apparent that a new mutant is in the third-chromosome it is out-crossed to a fly carrying both Dichæte and Hairless in the same third-chromosome homologue. The F_1 Dichæte Hairless females are then back-crossed by males of the mutant type. The results of such three-point back-crosses leave no doubt as to the position of the new mutant with reference to the two known loci. It is then often desirable or necessary to make a more refined study of the relation between the locus of the mutant and the loci of whatever other mutants are in the same region as the mutant.

TILT (tt).

(Figure 16, page 135.)

ORIGIN OF TILT.

In looking over the stock of black pink bent, Bridges found (August 29, 1915) several flies whose wings were held out at a wider angle from the body

than usual for that stock. The wings were tilted up at the ends, much as in the case of the second-chromosome mutant jaunty. Also, there was a long break in the third longitudinal vein in the region opposite the posterior cross-vein. Such a break had never been observed in the third longitudinal before,

FIG. 16.—Tilt wings. Note break in third-longitudinal vein.

and, in addition, the presence of the tilt and the abnormal wing-angle made it probable that a new mutation had arisen in the black pink bent stock.

CHROMOSOME OF TILT.

The tilt flies were at the same time homozygous for the three recessive mutants black (II–chromosome), pink (III), and bent (IV), and this fact made it exceptionally easy to determine in which chromosome the gene for tilt lies. A tilt male was out-crossed to a wild-female. In F_1 there were produced only wild-type flies, which showed that tilt is recessive. Two F_1 pairs were mated. Back-crosses could have been made, since other tilt flies were on hand, but it was feared that the presence of another wing character, viz, bent, would interfere somewhat with the accuracy of the classifications of tilt, and on account of the small size of the fourth chromosome there was relatively little probability of the new mutant being in the fourth chromosome with bent. A back-cross would have given no flies free from bent, and it was forseen that it would be desirable to eliminate bent and also either black or pink, depending

on whether the gene for tilt were in the third or the second chromosome. In F_2 the tilt character reappeared as approximately a fourth of the flies, nearly all of the tilt flies being at the same time pink. This linkage of tilt with pink showed that the gene for tilt is in the third-chromosome. Along with the linkage of tilt and pink was a random distribution of tilt and sex, and black, and bent.

TILT PINK BACK-CROSS.

From the F_2 flies a tilt pink male that was not black or bent was out-crossed to a wild female. Other similar males and females were crossed to each other

TABLE 89.—*P_1, tilt pink ♂ × wild ♀; B. C., F_1 + ♀ × tt p ♂.*

Oct. 20, 1915.	tt p	+	tt	p
2,274.....	66	45	8	7
2,321.....	103	93	6	7
2,322.....	161	145	11	13
Total....	330	283	25	27

to give a tilt pink stock as free as possible from these other characters. F_1 females were back-crossed to tilt pink males from this stock (table 89). The three cultures produced an aggregate of 665 flies, of which 52 or 7.8 per cent were recombinations for tilt and pink.

TILT PINK × DICHÆTE BACK-CROSS.

In order to determine whether the locus of tilt is to the left or to the right of that of pink, it was necessary to find the linkage relation of tilt to some other locus in that neighborhood. For this purpose Dichæte, which had just

TABLE 90.—*P_1, tilt pink ♂ × Dichæte ♀; B. C., F_1 D ♀ × tt p ♂.*

Nov. 20, 1915.	tt p	D	tt D	p	tt	D p	tt D p	+
2,494.....	67	90	0	0	4	3	0	0
2,495.....	80	79	0	1	6	5	0	0
2,496.....	84	95	0	0	9	5	0	0
Total....	231	264	0	1	19	13	0	0

been discovered and placed, was used. Both single mutant types were easily distinguishable from each other and from the wild-type. The tilt in the tip of the wing and the break in the third longitudinal were depended upon to show which Dichæte flies were at the same time tilt. None of the back-cross flies (table 90) were both tilt and Dichæte, which is an indication that the locus of tilt is very close to that of Dichæte, rather than to the right of pink. That the locus of tilt is slightly to the left of Dichæte is shown by the single

not-tilt not-Dichaete pink fly that appeared. This one tilt Dichaete recombinations in a total of 3048 flies means that the locus of tilt is about 0.3 unit to the left of Dichaete, or at 40.4 if referred to ruby=0.0 as zero.

DESCRIPTION OF TILT.

Part of the spreading of the wings observed in the first tilt flies was due to the bent character that was also present. When the bent was eliminated, the tilt wings were found to be held out in the majority of cases. In about a quarter of the flies there is only slight or even no divergence of the wings; in another quarter there is divergence up to about 30° in each wing; in the remaining half the divergence ranges up to a full right angle for each wing. There seems to be rather a sharp division between these extreme divergents and the slight or normal type; whether this difference is genetic or fluctuating has not been tested. The break in the third longitudinal vein also was found to be variable. About 90 per cent of the tilt flies have a marked break in one or both wings; in the remaining 10 per cent there is a weakening of the vein and usually a succession of small breaks with weak sections between. With careful examination the tilt flies can all be separated out by means of the vein character. The tilt characteristic is constantly present in all tilt flies, and is only slightly subject to fluctuation in the degree of tilting. In making the separations of tilt versus not-tilt, the flies are first sorted over rapidly with attention to the striking wing divergence of the tilt character. Then the remaining tilt flies are separated out, and both classes checked, by means of the tilt and the break. The speed of separation is somewhat increased by this method, which is used in the case of Dichaete, of Gull, of Expanded, of balloon, of bifid, of fused, and indeed in all cases in which a mutant is characterized by a striking but not invariable character and by an inconspicuous but constant difference.

EVALUATION OF TILT.

Tilt fills a place in the third-chromosome analogous to that of jaunty in the second. Both are excellent characters—viability, fertility and productivity normal, separations certain and rapid—but both have the misfortune to be only about 0.3 unit from the most-used mutants in their respective chromosomes. In nearly all problems black would be used in preference to jaunty and Dichaete in preference to tilt. There remain certain problems, such as dichismy, that require the most intensive study of particular regions, and both jaunty and tilt are retained for such contingencies, and not because of general usefulness.

BITHORAX (bx).

(Figure 20a, page 152; Figure 17, page 138.)

ORIGIN OF BITHORAX.

A mutant eye-color like maroon had been found by Bridges, and a male had been crossed to a female from the "New York" wild stock. In F_2 the color reappeared, but was so ill-defined that it was discarded as worthless. However, approximately a quarter of the flies showed (culture 2203, September 22, 1915) a new character of a surprising nature. These flies appeared to have

two thoraxes with wings and bristles complete. Behind the normal thorax, in the cleft between the thorax and abdomen, was inserted a second thorax, in which the parts of the normal thorax were recognizable, though smaller, much shortened, and otherwise distorted. Some of these "bithorax" flies were mated together, and all the progeny were found to be bithoracic, constituting a pure-breeding stock of the recessive mutant.

DESCRIPTION OF BITHORAX.

The mutant bithorax is characterized by a more or less complete change of the normal metathorax into a segment like the normal mesothorax. The extent of this change is variable, and often unequal on the two sides of the same individual. The factors (probably part at least environmental) that

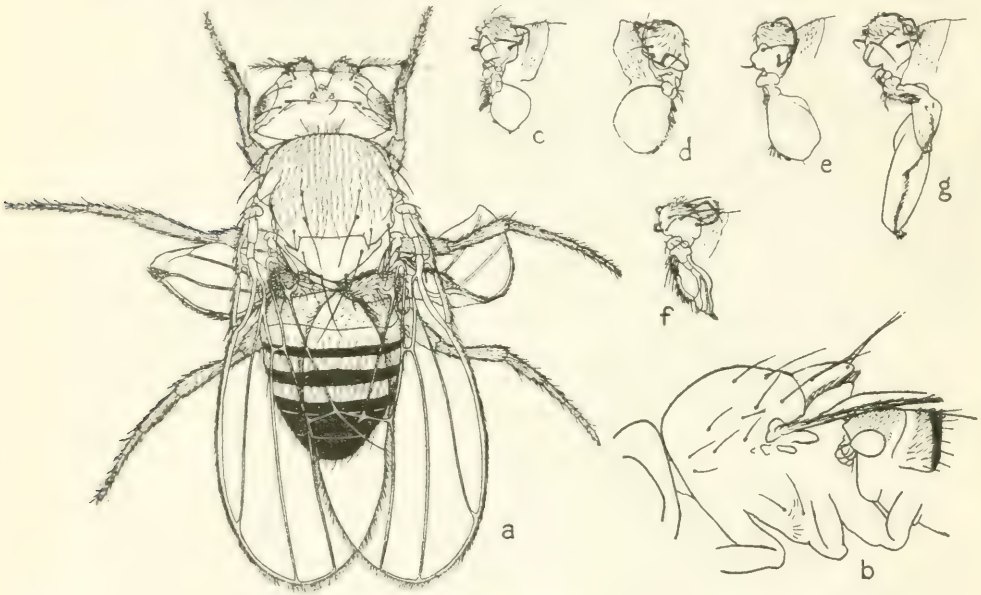


FIG. 17.—Bithorax. (a) bithorax glass, the extra wings especially clear as to venation. (b) normal balancer. (c, d, e, f, g) various bithorax balancers that are only slightly wing-like.

influence the extent of the change have not been investigated. In very rare cases the gene fails to produce any observable effect. Since in extreme fluctuants the bithorax character may show only on one side, both sides of all flies that appear at first glance to be not-bithorax must be examined if the classification is to be accurate. Nearly always a bithorax fly can be recognized at once by the presence, on one or both sides, of what looks like a black lump between the metathorax and the abdomen, just under and behind the base of the wing. More careful examination shows this "lump" to be a hairy structure that seems to grow over more or less of the dorsal surface of the metathorax. This dorsal hairy structure usually bears bristles, and shows a constriction that evidently corresponds to that between the normal mesonotum and scutellum.

Bristles corresponding to all the normal mesonotal bristles have been identified at one time or another. Bristles corresponding to the sternopleurals have also been found on the metapleura; but no sutures have been seen on the metapleura.

The balancers of bithorax are nearly always conspicuously modified. They are usually directed downwards instead of up and backwards as in the wild-type fly. They are also usually swollen, darkened, and hairy. The larger ones when inflated suggest the well-known "balloon" wings. Sometimes they are not inflated, but flattened and wing-like. In these cases veins can be made out. The larger balancers are apparently always covered with minute hairs, like those on the surface of the wings. What is normally the outer anterior surface of the balancer usually bears, in bithorax, a row of black hairs evidently corresponding to the heavy black hairs along the costal vein of the normal wing. Study of these hairs in various types of bithorax balancers leads to the conclusion that the constrictions separating the normal balancers into three parts correspond to the two costal breaks in the normal wing (one at the humeral cross-vein, the other just before the tip of the first longitudinal vein). In the best-developed specimens, the extra wings are about half as large as normal wings, and the venation is clearly like that of the normal wing.

The third leg of a wild-type *Drosophila melanogaster* differs from the second leg in two respects: The tibia of the second leg has an apical and a preapical bristle; there is only a preapical on the third. The basal tarsal joint of the third leg bears at its base, on the under side, a few bristle-like hairs that are distinctly longer than the corresponding hairs on the basal tarsal joint of the second leg. The third leg of bithorax flies frequently shows an apical tibial bristle, but it is more curved than the apical tibial bristle of the normal second leg. It bears a striking resemblance to the "hind tibial spur" of the genus *Hippelates*. The hairs of the basal tarsal joint of the third leg have not been found to be shorter in bithorax flies than in wild-type ones.

BITHORAX × STAR.

From the pure stock of bithorax, males were out-crossed to the second-chromosome dominant Star. None of the flies from this cross showed the bithorax character, which was thus definitely proved to be recessive. An F_1 Star male was back-crossed to a bithorax female. Of the 200 flies produced by this male back-cross test (table 91) 108, or 54 per cent, were either Star bithorax

TABLE 91.—P_1, *bithorax* ♂ × *Star* ♀; *B. C.*, F_1 *S* ♂ × *bx* ♀.

Oct. 30, 1915.	S	bx	S bx	+
2,353.....	47	45	55	53

or wild-type. The presence of these classes was due to the free assortment for the genes for Star and for bithorax, and showed that the gene for bithorax is not in the second-chromosome.

BITHORAX × SEPLE

From a cross of bithorax to "seple" (se ss e^s ro), F_1 wild-type males and females were bred together. In F_2 none of the bithorax flies showed any of the characters that entered the cross from the seple parent. This proved that the gene of the new mutant is in the third-chromosome.

THE APPROXIMATE LOCATION OF BITHORAX AS FOUND BY TESTS OF SEPLE CROSS-OVERS.

In the F_2 from the cross of bithorax to seple there occurred flies that showed one or another cross-over combinations of seple, such as sepia, sooty rough, or spineless sooty rough. Males that represented crossing-over in various sections were out-crossed to bithorax females. The sepia males that were tested were found to have a sepia bithorax chromosome; that is, the gene for bithorax is situated to the right of the cross-over region between sepia and spineless. Likewise the sooty rough cross-overs carried bithorax, which showed that the bithorax locus is to the left of the cross-over region, between spineless and sooty. None of the flies with any combination including spineless itself were found to carry bithorax. The limits for the locus of the bithorax gene were thus proved to be within a few units to the right or to the left of the spineless locus.

Stocks of sepia bithorax and of bithorax sooty rough were obtained from the descendants of the above tests. These two stocks were crossed together, and, by further crossing-over, a stock of sepia bithorax sooty rough flies was secured. This stock was called "seble."

SEPIA BITHORAX × HAIRLESS.

At this time the third-chromosome dominant Hairless had been found and its locus determined as approximately a unit to the left of ebony. It was recognized that Hairless would become one of the most useful third-chromosome mutants, and would be suitable for determining the locus of new mutants such as bithorax. A sepia bithorax female was crossed to a Hairless male, and F_1 Hairless females were back-crossed by sepia bithorax males (table 92). There

TABLE 92.—*P_1, sepia bithorax ♀ × Hairless ♂; B. C., F_1 H ♀ × se bx ♂.*

Sept. 22, 1916.	se bx	H	se H	bx	se bx H	+	se	bx H
5,274.....	83	106	27	48	13	17	5	9
5,275.....	41	47	30	37	6	12	4	2
5,305.....	43	33	20	26	5	10	3	2
5,306.....	49	62	29	27	8	7	5	3
Total....	216	248	106	138	32	46	17	16

was 13.6 per cent of recombination for bithorax and Hairless, and since from other experiments there was about 10 per cent of recombination for spineless and Hairless, the locus of bithorax was supposedly slightly to the left of that of spineless.

SEBLE × DICHÆTE HAIRLESS: CROSS-OVER VARIATION PRESENT.

This position of the bithorax gene was regarded as favorable, since the mutant bithorax could be used as an alternate to spineless. The positions of these two loci were approximately the same, the exact distance between being determinable by further experiments; and the character bithorax was of such a nature that it could be used with any known type of character without interference with the accuracy of classification, while the bristles of the spineless mutant were so small that it would be difficult to use spineless with other mutants that also affect size of bristles.

A Dichæte Hairless double-dominant stock was made up; and a Dichæte Hairless male was crossed to a sepia bithorax sooty rough male. The F_1 Dichæte Hairless females were back-crossed by se bx e^s ro males. About 1,000 back-cross flies were raised (table 93) of which 85 or 8.2 per cent represented

TABLE 93.—*P_1, sepia bithorax sooty rough ♀ × Dichæte Hairless ♂; B. C., F_1 D H ♀ × se bx e^s ro ♂.*

	0		1		2		3		4		5		1,2		1,3		1,4		1,5		2,5		3,5	1,2,3
Nov. 23, 1916.	se bx e^s ro	D H	se D H	bx e^s ro	se H	D bx e^s ro	se bx H	D e^s ro	se bx	D H e^s ro	se bx e^s ro	D H ro	se D bx e^s ro	H	se D bx e^s ro	H	se D H e^s ro	bx	se D H e^s ro	bx	se H e^s ro	D bx	se bx H	e^s ro
46	73	65	6	12	13	5	8	11	1	0	27	9	1	0	1	1	1	0	2	2	1	0	0	0
54	53	50	3	9	6	2	4	1	0	4	22	17	1	0	0	2	1	0	3	0	0	1	0	1
56	39	28	1	6	3	3	5	5	0	0	8	4	0	0	0	0	0	0	2	2	1	0	0	0
57	69	51	8	4	8	5	8	9	2	3	23	15	0	0	0	1	1	0	0	1	0	1	1	0
87	16	11	3	4	5	1	4	3	0	0	6	5	0	0	0	1	0	0	0	0	0	0	0	0
88	42	22	6	5	8	2	6	3	1	0	14	11	0	0	0	0	0	1	1	0	1	0	0	0
89	43	32	5	3	4	3	6	3	0	0	11	7	1	1	0	1	0	0	2	1	0	1	0	0
Total	335	259	32	43	47	21	41	35	4	7	111	68	3	1	1	6	3	1	10	6	3	3	1	1

recombination for bithorax and Hairless. The recombination per cent for spineless and Hairless was found, from other experiments, to be approximately 11, and a comparison of these values showed that the locus of bithorax is probably between spineless and Hairless, that is, 1 or 2 units to the right of spineless. The se bx × H back-cross (table 92) had seemed to show that the locus of bithorax was 2 or 3 units to the left of spineless, rather than to the right. But the present experiment was on a larger scale than the former, and seemed more trustworthy. Considering both experiments together, the mean position for the bithorax locus was about a unit to the right of spineless.

The sepia Dichæte recombination per cent was 10.3 and the Dichæte bithorax per cent 7.6, which values agreed with the previously found values for se D and D ss. But soon the results of other experiments showed that values around 14 and 15, respectively, for these two sections were common. In a given experiment the values for the two sections were usually both low or both high, while the values for the region to the right of spineless gave the same values in both cases. From this Bridges inferred that in certain stocks a cross-over variation was present that reduced the crossing-over in the region to

the left of spineless. More recent work leads to the conclusion that the effect is upon the left limb of the third-chromosome, but not upon the right, offering a parallel to the regional action of CHL and CHR upon the second-chromosome and of CH upon the third. These cross-over variations are discussed more at length in special sections (CHIP on p. 183, CH on p. 89). That the variation was introduced through the Dichæte Hairless rather than the "seble" stock is indicated by the normal values obtained in a seble back-cross carried out in connection with divergent (table 94).

TABLE 94.—*P_1, sepia bithorax sooty rough × divergent; B. C., F_1 + ♀ × se bx e^s ro ♂.*

Nov. 5, 1917.	se bx e^s ro	+	se	bx e^s ro	se bx	e^s ro	se bx e^s	ro	se	bx	se	bx e^s ro	se e^s	bx ro
8,023....	70	65	37	30	5	9	16	19	6	4	5	6	1	1
8,024....	53	60	35	32	13	6	11	16	3	4	6	4	..	..
8,025....	51	61	19	22	6	10	14	10	..	4	8	5	..	..
Total..	174	186	91	84	24	25	41	45	9	12	19	15	1	1

A COMPARISON OF BITHORAX HAIRLESS AND SPINELESS HAIRLESS RECOMBINATION PERCENTAGES OBTAINED IN SISTER CULTURES—THE PARALLEL BACK-CROSS METHOD.

The occurrence of genetic cross-over variations, and the fact that crossing-over is affected by age and by temperature, make any conclusion drawn from a comparison of values from different experiments subject to doubt. A method of escape from this difficulty lies in using sister flies for the two experiments and raising the cultures simultaneously with like culture treatment throughout. Thus, in finding the relative order of bx and ss it was decided to cross bithorax sooty to spineless, and to back-cross several F_1 wild-type daughters to bithorax sooty, at the same time back-crossing an equal number to spineless sooty. The first set of cultures give a bithorax sooty recombination percentage and the second set give a comparable spineless sooty percentage. Because the simple stocks mentioned above were not on hand, the actual cross was se bx e^s ro ♂ × ss (wo) ♀; and the F_1 females were tested by se bx e^s ro on the one hand and by se ss e^s ro on the other (tables 95 and 96). The bx e^s percentage was 11.8, based on 2,374 flies; while the ss e^s percentage was 13.6, based on 2,329 flies. The locus of bithorax is therefore probably to the right of that of spineless by somewhat less than 2 units. Similarly, the se bx per cent was 26.8, slightly greater than the comparable se ss per cent of 26.1. These two comparisons agree in placing bithorax slightly to the right of spineless. The reference to sepia is less accurate than that to sooty, because sepia is farther away from the region under test than is sooty.

The principal advantage of the parallel back-cross tests is to furnish information on the basis of which a more direct method can be undertaken.

A three-point ss bx H back-cross is, of course, much more accurate in measuring the interval between ss and bx than the parallel tests, but with loci as close together as these it is very difficult indeed to obtain the necessary double recessive ss bx without a special method. The special method consists in first determining between which two established loci the new locus is situated, and then testing cross-overs between these primary loci, a certain proportion of these cross-overs being cross-overs between a given primary locus and the new locus. A fly coming from such a cross-over would have a chromosome carrying the

TABLE 95.—*P_1 sepia bithorax sooty rough ♂ × spineless (white-ocelli) ♀; B. C., F_1 + ♀ × se bx e^s ro ♂.*

Mar. 15, 1920.	se bx e^s ro	+	se	bx e^s ro	se bx	e^s ro	se bx e^s	ro	se e^s ro	bx	se	bx e^s	se bx ro	e^s	se e^s	bx ro
11,519	60	136	17	48	8	22	18	39	6	11	11	10	1	2	..	..
11,520	90	103	36	30	18	14	33	35	5	7	16	11	1	..	.	..
11,521	96	108	36	37	19	11	36	36	5	6	11	15	..	2	..	..
11,522	87	89	37	30	14	18	31	27	6	5	6	6	3	.	1	..
11,523	56	119	24	57	7	28	15	47	5	9	12	17	1	..	.	..
11,524	114	108	35	42	17	25	20	25	2	2	11	11	..	..	..	..
Total	503	663	185	244	82	118	153	209	29	40	67	70	6	4	1	..

TABLE 96.—*P_1, sepia bithorax sooty rough ♂ × spineless (white-ocelli) ♀; B. C., F_1 + ♀ × se ss e^s ro ♂.*

Mar. 15, 1920.	se e^s ro	ss	se ss	e^s ro	se	ss e^s ro	se e^s	ss ro	se ss e^s ro	+	se ss e^s ro		se ss ro	e^s	se ss e^s ro	ss e^s	se ss e^s	ro
11,514	131	126	50	40	31	32	50	35	7	7	11	14	3	1	1	..		
11,515	125	117	41	36	21	21	32	39	4	9	15	20	1	2	1	..		
11,516	94	87	46	34	14	17	34	40	6	4	11	15	2	2	..	..		
11,517	97	94	30	41	20	19	40	33	7	8	10	19	2	..	1	1		
11,518	97	122	43	38	29	20	47	38	6	8	9	12	5	1	1	2		
Total	544	546	210	189	115	109	203	185	30	36	56	80	13	6	4	3		

genes for the primary mutation and for the new mutation, and from it a double recessive stock could be obtained. In the case of bithorax, it was regarded as established that the locus of bithorax lies between those of spineless and sooty. A peach spineless sooty rough male ("peple") was crossed to a bithorax female, and F_1 females were mated to other peple males. Since bithorax was supposed to lie about a unit to the right of spineless and about 10 units to the left of sooty, approximately one-eleventh of the p^p ss not-e^s not-ro flies were expected to carry a p^p ss bx chromosome. Such p^p ss cross-over males were tested for the presence of bx by crossing to bx females. Any bithorax fly produced by such matings would be bx in one third-chromosome

and p^p ss bx in the other, and homozygous p^p ss bx flies would be produced by crossing them together. Since finding a p^p ss bx chromosome is the only point to be considered the tests could be advantageously carried out by mating about 3 p^p ss males to about 5 bx females per culture. Several such mass tests were carried out; but none of them produced bx flies. Either not enough tests were started, or the locus of bithorax is closer to that of spineless than supposed.

LOCATION OF BITHORAX BY USE OF GLASS.

The weakness of the situation was that sooty was so far away from the region in which the cross-over was desired that only about 1 in 11 of the flies representing crossing-over were expected to be of the right sort. No other satisfactory mutants were present in the sections, by use of which the proportion of waste cross-overs could be cut down appreciably. Somewhat later, the mutant "glass" was found, and was located at about 5 units to the right of spineless. A spineless glass male was crossed to a bithorax female, and the F_1 females were back-crossed to spineless glass males. About 1 in 5 of the spineless not-glass flies produced were expected to be ss bx in one chromosome. But as a matter of fact only about 1 in 30 was such a cross-over. The occurrence of this one cross-over proved that the locus of bithorax is really to the right of that of spineless; and also gave a means of getting a spineless bithorax glass triple recessive.

SPINELESS BITHORAX GLASS BACK-CROSSES.

Three types of three-point back-crosses involving spineless bithorax and glass were carried out (tables 97, 98, 99). In all three experiments the bithorax

TABLE 97.—P_1, *spineless glass*$\times$*bithorax; B. C.,* $F_1+\varphi\times$*ss bx gl* σ.

March 27, 1921.	ss gl	bx	ss bx	gl	ss	bx gl
12,608	155	201	2	..	3	6
12,609	204	220	..	1	16	10
12,618	184	163	1	1	5	8
12,619	145	155	..	1	1	10
12,650	171	167	..	1	8	6
12,682	133	144	..	1	7	8
Total	992	1,050	3	5	40	48

TABLE 98.—P_1, *spineless bithorax*$\times$*glass; B. C.,* $F_1+\varphi\times$*ss bx gl* σ.

April 28, 1921.	ss bx	gl	ss gl	bx	ss bx gl	+
12,733	125	116	..	..	6	4
12,734	118	119	..	..	1	5
12,735	124	134	..	..	3	7
12,736	142	168	..	..	6	12
12,737	112	128	..	..	5	6
12,738	123	145	..	..	8	3
Total	744	810	..	..	29	37

TABLE 99.—*P_1, spineless × bithorax glass; B. C., F_1 + ♀ × ss bx gl ♂.*

May 15, 1921.	ss	bx gl	ss bx gl	+	ss gl	bx
12,801	74	61	..	1	3	4
12,802	88	97	..	1	3	2
12,803	78	61	..	..	1	..
12,810	100	118	1	1	7	9
Total	340	337	1	3	14	15

glass value was 4.1. In the first experiment the spineless bithorax recombination per cent was 0.4, in the second 0.0, and in the third 0.6. The mean value for spineless bithorax is thus 0.3.

SPINELESS BITHORAX × WILD BACK-CROSS.

A simple stock of bithorax was needed in building a certain multiple stock, when it was found that the simple stock had been discarded as an economy in stock-keeping. Accordingly, a bithorax fly free from spineless was sought in a ss bx × wild back-cross (table 100). Only one recombination appeared

TABLE 100.—*P_1, spineless bithorax ♂ × wild ♀; B. C., F_1 + ♀ × ss bx ♂.*

June 20, 1922.	ss bx	+	ss	bx
13,163	207	192	..	..
13,164	206	234	1	..
13,165	184	229	..	..
13,166	178	198	..	..
13,171	187	194	..	..
13,172	87	116	..	..
Total	1,049	1,163	1	..

in a total of 2,213 flies, and this was the spineless, not the bithorax recombination. Another experiment of like nature in connection with stripe (table 203) gave a similar result. These experiments indicate that the locus of bithorax is closer to that of spineless than was supposed. It was noticed also that the recombination class that was not-bithorax was represented by several flies, and it seemed probable that some of these were rare "changed-over" bithorax original combinations rather than not-bithorax recombinations. In still a further experiment tests were made of some of the supposedly not-bx recombinations, and many of them were found to be genetically homozygous bithorax.

EVALUATION OF BITHORAX.

The mutant bithorax is chiefly interesting in the light that it throws on the homologies between the structures of the normal balancer and metathorax as

compared with the different structures of the wing and mesothorax. Genetically considered, bithorax is an alternate to the more useful locus spineless; and will probably be used mostly in combination with characters like Minute that can not be used successfully with spineless. The character overlaps the wild-type to a very slight degree, but this is enough to prevent its further use in experiments in which small percentages are being tested.

SMUDGE (sm).

(Figure 18.)

ORIGIN OF SMUDGE.

A stock of the dominant third-chromosome mutant Dichæte was observed by Bridges to have as an impurity a "moruloid" eye character (November 5, 1915). The facets of the eyes of these flies were disposed somewhat irregularly, as in the case of morula or Star, but the disturbance was not very marked.

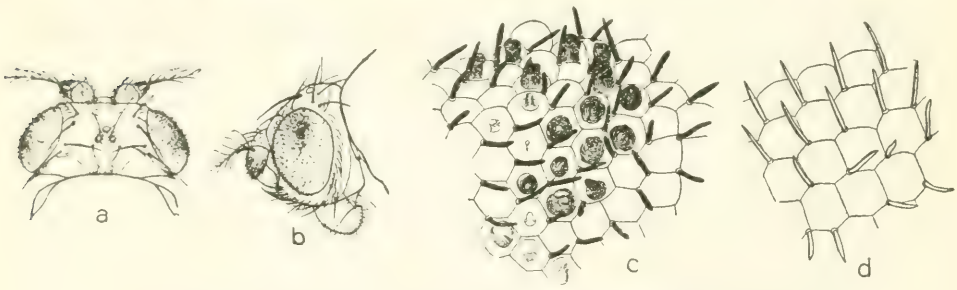


FIG. 18.—Smudge eyes. (a and b) top and side view of smudge eye. (c) facets and hairs of the smudge area. (d) facets and hairs of the not-smudge area of the smudge eye.

With a magnification of 38 diameters (the routine examinations are made with a magnification of about 15 diameters) it was seen that quite a few of the flies that had not seemed moruloid were really so. The facets in small areas were found to be rounded instead of hexagonal.

The new mutant character did not seem very workable because of the labor and uncertainty involved in its separations. It was therefore decided to discard the mutant, and also to eliminate it from the Dichæte stock, so that it would not become spread more widely by crosses in which Dichæte would be used. Pair-matings were made between Dichæte flies that did not show the moruloid. From those pairs that did not produce moruloid among the offspring, further matings were made, and this process was repeated until the stock was free from the impurity.

One of the first of the selected pairs produced moruloids, and also flies that had a black smudge on the upper part of the compound eye (2466, November 18, 1915).

DESCRIPTION OF SMUDGE.

The smudge varied from a few black granules here and there on the upper part of the eye to a patch half the area of the eye. Under low-power the eye looked as though smudged with soot or pencil lead. Under high-power the black granules were seen to be individual ommatidia that were somewhat enlarged and much darkened. There was also a cone-shaped eruption of the surface of the black ommatidia. In the smudge area the facets tended to be irregularly distributed and to be rounded. The hairs set at the corners between the facets were sometimes found to be blacker, shorter and rather thicker. Because of the disturbance to the rows of ommatidia, these hairs were often in irregular groups. The eye-color was often darker than normal. When smudge males and females were bred together a stock was obtained in which roughly 80 per cent of the individuals were seen to have the smudge to some degree, while the remainder were mostly moruloid.

CHROMOSOME OF SMUDGE.

To test whether the locus of smudge is in the second chromosome, smudge males were crossed to vestigial females, and five F_2 pair-cultures were raised

TABLE 101.—P_1, *smudge* ♂ × *vestigial* ♀ : F_2, F_1 + ♀ × F_1 + ♂.

Feb. 1, 1916.	+	vg	sm	vg sm
3,055	47	17	18	4
3,056	36	15	11	2
3,057	40	14	26	6
3,871	33	16	20	4
3,872	25	14	23	4
Total*	181	76	98	20

* Two other cultures gave very few vestigials, because of a recessive second-chromosome lethal found (by L. J. Cole) to be in our vestigial stock. (Culture 3194 gave 119+, 2 vg; sister culture 3195 gave 135+, 5 vg.)

(table 101). In the F_2 cultures vestigial smudge flies occurred, and the ratio appeared to be the 9 : 3 : 3 : 1 ratio typical of free assortment, from which it was concluded that the locus of smudge is not in the second chromosome and is therefore probably in the third.

LOCUS OF SMUDGE—SMUDGE × DICHÆTE HAIRLESS BACK-CROSS.

The distribution of smudge and Dichæte observed in the stock should then mean that the locus of smudge is rather far distant from that of Dichæte. At that time it was thought that sepia was at the left end of the chromosome, and since the sepia Dichæte distance was about 10, the locus of smudge was supposed to lie to the right of Dichæte and as far distant as Hairless. By the simultaneous use of Dichæte and Hairless the relative order of smudge and its distance from the nearest of the two dominants could be determined.

When a smudge by Dichæte Hairless back-cross was carried out, considerable difficulty was met with in classification. There seemed far too many smudge

flies present (a slight excess of smudge had appeared in the smudge by vestigial F_2), and these were distributed with respect to Dichæte and Hairless in such a way that the position of smudge was very problematical. The counts were finally abandoned and the character smudge was put aside as of little value.

Later, in looking over these results, a possible solution of the difficulty presented itself. It was recalled that all flies had been classified as smudge if they showed the granules or if they showed the irregular arrangement of facets and general darkening of color. It then seemed probable that there were present two distinct mutant types—the moruloid character originally observed, and the true smudge that was noticed in the next generation and was supposed to be another and more extreme effect of the same mutant gene. Possibly these characters were interrelated, for example, in such a fashion that one appeared only in flies homozygous for the gene of the other. A second smudge × Dichæte Hairless back-cross was undertaken, and in making the separations only those flies were classified as smudge in which the black-granular ommatidia were

TABLE 102.—*P_1, smudge ♂ × Dichæte Hairless ♀ *; B. C., F_1 D H ♀ × sm ♂.*

Nov. 4, 1920.	+	D	H	D H	sm	sm D	sm H	sm D H	% sm
12,084.....	84	35	36	110	44	7	9	5	19.7
12,085.....	87	35	43	123	34	10	10	6	17.2
12,086.....	61	41	50	103	26	6	5	3	13.6
12,087.....	56	35	19	87	48	10	17	9	29.9
12,088.....	45	38	26	97	71	7	8	12	32.2
12,141.....	30	20	12	46	36	4	8	7	33.7
12,142.....	32	37	21	58	36	13	22	17	37.3
12,179.....	22	26	22	53	69	9	36	20	52.1
Total....	417	267	229	677	364	66	115	79	.....

* The P_1 mating produced: 99 D H, 138 +, 38 D, 54 H; 12,047.

observed. The ordinary low power was used, and probably the separation of the smudge from the not-smudge was incomplete (table 102, cultures 12084 to 12088). Three of the cultures (12084, 12085, 12086) gave results that seem similar, with 17.0 as mean percentage of smudge. The other two (12087, 12088) gave a higher percentage with a mean of 31.1. These two kinds of results seem significantly different, and probably mean that the inheritance of smudge is digenic, and that the smudge P_1 fly was heterozygous for one of these genes. Three additional cultures were raised from the high-type cultures, and these (12141, 12142, 1879) were all of the high type. These last three cultures showed that the locus of the third-chromosome gene is to the left of Dichæte by a long interval, which is not exactly determinable because of the digenic nature of the smudge character. A position near 15 seems probable. The presence of the vestigial smudge flies in the F_2 of table do not exclude a dominant second-chromosome gene requiring the simultaneous action of the third-chromosome gene to give the vestigial smudge flies. To be certain that the above interpretation of the inheritance of smudge is correct would require rather extensive tests, and it did not seem worth while to carry these out,

since the principle involved is already established by other cases thoroughly analyzed.

SKI-III (si).

(Figure 19.)

The mutant Ski has been proved to depend upon the simultaneous action of a dominant second-chromosome gene and a recessive third-chromosome gene. In this account we are concerned primarily with the third-chromosome

FIG. 19.—Ski wings (simplex).

gene; but a short general account of the discovery of the character and also of its digenic nature is abstracted from a forthcoming paper by Clausen and Collins.

ORIGIN OF SKI.

The mutant-type Ski was found by Clausen and Collins to be present in a small proportion of the flies in two sister F_2 mass-cultures that were descended from a cross of white to wild (December 1, 1915). The Ski flies were bred together, and a stock was produced which threw a high proportion of Ski flies. The stock did not breed true for the character even after considerable selection. Matings within the stock showed that the wild-type flies mated together never gave Ski; that Ski × Ski gave 3 Ski : 1 wild-type, or more rarely, all Ski; and that Ski × wild-type gave 1 Ski : 1 wild-type, or sometimes, all Ski.

Several attempts were made to determine the locus of Ski, but these gave discordant results. Clausen and Collins made test matings which showed that the inheritance of Ski was remarkably similar to that of vortex, which had just been worked out by Bridges and Mohr (Genetics, 1919). That is, Ski is dependent on the simultaneous presence of two genes, neither of which gives any somatic effect by itself. One of these genes (Ski–III) is located in the third chromosome at about 5 units to the right of Dichæte; and the

other (Ski–II) is located in the second-chromosome about 13 units to the left of black. The Ski flies were found to be of two types: all were homozygous for ski–III, but they could be either heterozygous or homozygous for Ski–II. They established, by rigorous methods, stocks homozygous for both genes, which stocks bred true to the Ski character. The pure-breeding or "duplex" Ski was found to be similar to but somatically distinct from the "simplex" Ski.

DESCRIPTION OF SIMPLEX AND DUPLEX SKI.

The character Ski, simplex, is almost identical with jaunty; that is, the wings are turned up at the tips, usually only the posterior half of the wing. In duplex Ski the curvature usually involves the whole wing, and is more extreme, the tip often being directed forward. In duplex Ski there is a strong infolding or crimp in the costal vein, which is only slightly developed in simplex Ski, and which is not present in jaunty or curled at all. There is considerable overlap between simplex and duplex Ski, but none between Ski and the wild-type. In this respect Ski is much more sharply defined than the similarly inherited vortex, which fluctuates markedly, is sensitive to modification, and is sex-limited in expression.

Clausen and Collins showed that the ratio of wild-type to Ski produced in various matings had the following unusual range of values: +: Ski = 7: 1, 13: 3, 3: 1, 5: 3, 1: 1 or 1: 3. In one mating of Ski × wild-type (duplex Ski × ski–III) the F_1 are all Ski; and F_2 is 3 Ski: 1 wild-type, which represents the usual 1: 2: 1 ratio of a simple *dominant*. Another mating of Ski × wild-type (duplex Ski × Ski–II), the F_1 is wild-type; and F_2 is 3 wild-type: 1 Ski, corresponding to the 1: 2: 1 ratio of a simple *recessive*.

Two wild-type stocks (Ski II and Ski III) mated together give only wild-type F_1; and yet in F_2 Ski appears in 3 of the 16 classes. Curious linkage discrepancies were also found, making the character appear sometimes as a simple III-chromosome recessive and again as a simple II-chromosome dominant.

COMPLEMENTARY BACK-CROSSES OF DICHÆTE, SKI-III, AND SPINELESS.

Since the viability of Ski had been observed to be inferior to that of the wild-type under the conditions of the experiments, the four complementary

TABLE 103.—*B. C. data involving Dichæte, ski*-III, *and spineless.*

Feb. 28, 1920.	"0"	"1"	"2"	"1, 2"	Tot.	D-si	si-ss	D-ss
D si ss × + . .	2,936	109	280	17	3,342	3.8	8.9	11.6
D × si ss.....	2,501	137	339	31	3,008	5.6	12.3	15.8
D si × ss. . .	4,168	158	423	25	4,774	3.8	9.4	12.2
D ss × si.....	3,773	284	461	29	4,497	5.8	10.9	15.5
Total.....	13,378	688	1,503	102	15,621	4.7	10.2	13.7

types of back-cross were made (table 103). The combination of the data from these four crosses gives a neutralization of the effects of inviability. This method has the further great advantage that since the sources and relations

of the chromosomes are varied, cross-over variations are also neutralized or revealed. The results have, therefore, much greater reliability and generality.

In making the back-cross tests it was necessary that one or the other of the parents be homozygous for Ski II, while the male must be homozygous for ski III. Thus, one back-cross was of the type $\frac{Si\ D}{Si\ +}\ \mathcal{Q} \times \frac{+\ +}{+\ si}\ \mathcal{J}$, while another was of the type $\frac{+\ D}{+\ +}\ \mathcal{Q} \times \frac{Si\ +}{Si\ si}\ \mathcal{J}$. In both cases all the back-cross flies that receive the Ski gene from the mother are simplex Ski.

The locus of ski–III is between those of Dichæte and spineless. There was 5.7 per cent of recombination for Dichæte and ski–III, and 11.4 per cent for ski–II and spineless. In both of these sections there is double crossing-over, so that the map-distance corresponding to these recombination per cents are approximately 5.9 and 11.4.

The crosses of Ski to Star Dichæte, with subsequent mating of F_1 S D females by simplex Ski males (table 104) produced for the Dichæte–ski III interval

TABLE 104. *P_1, Ski × Star Dichæte; F_1 S D ♀ × simplex Ski ♂ (Aug. '19).*

Si	S Si	D Si	S D Si	+	S	D	S D
308	291	12	17	176	299	669	713

the equivalent of 40 recombinations in a total of 779, or 5.1 per cent of recombination.

The position finally assigned to ski–III on the basis of all the data is 46.5; but the relative order of ski–III with respect to the rest of the numerous loci in that immediate vicinity is still undetermined.

The coincidence was 134, which is the highest met with in any experiment involving the same Dichæte spineless interval. The high coincidence is to be explained on the same grounds as the massing of mutants in that region of the map, namely, that a unit of map-distance corresponds to a longer section of chromosome in that region than elsewhere—that the "coefficient of crossing-over" is low.

DILUTE.

ORIGIN OF DILUTE.

In determining the location of the new sex-linked recessive, cut, several F_2 cultures from the cross of cut to vermilion forked were raised by Bridges. In some of these cultures an eye-color mutation resembling maroon was observed (December 9, 1915, No. 2625). This mutant was present in approximately a quarter of the individuals in both males and females, and in all classes with respect to the sex-linked characters, so that it was known to be an autosomal recessive. The cut and the vermilion forked stocks were examined, and in the vermilion forked stock some "dilute" vermilions were found that

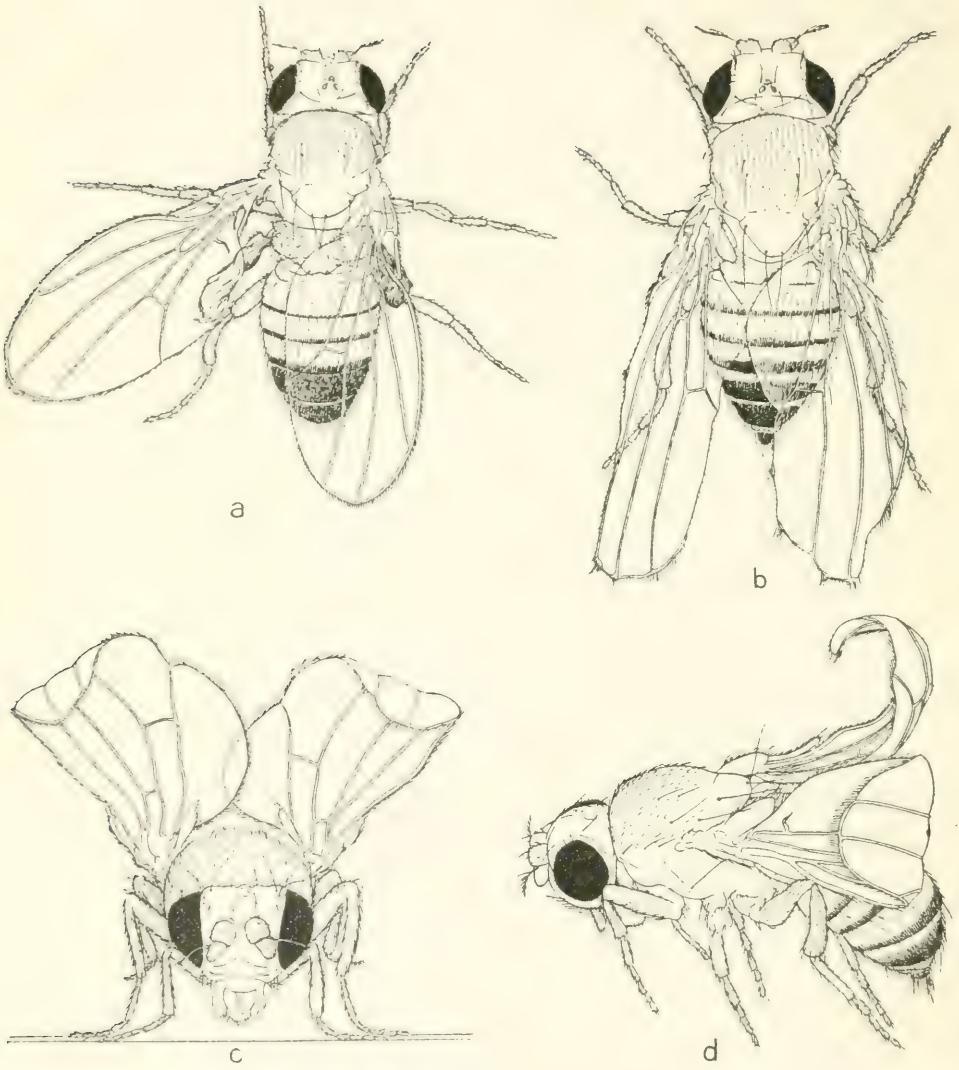


FIG. 20.—Third-chromosome wing mutants. (*a*) bithorax, showing balancers turned into extra wings; (*b*) typical Beaded of stock; (*c*) curled wing, front view; (*d*) curled wing, side view.

were apparently the same as the vermilion dilutes of the F_2 cultures. A pair of these vermilion forked dilutes gave a stock apparently pure.

DILUTE × DICHÆTE, MALE BACK-CROSS.

From the vermilion forked dilute stock a male was out-crossed to Dichæte, and an F_1 Dichæte male was back-crossed to a vermilion forked dilute female (table 105). In the back-cross progeny there were only half as many males as

TABLE 105.—*P_1, dilute ♂ × Dichæte ♀; B. C., F_1 D ♂ × dil. ♀.*

Jan. 21, 1916.	D ♀	dil. ♀	D dil. ♀	+ ? ♀	D ♂	dil. ♂	D dil. ♂	+ ? ♂
2,882.....	65	53	.....	25	23	29	.....	19

females (71 : 143), and this was attributed to a sex-linked lethal and disregarded. There was a deficit of Dichæte from the expected equality (D : + = 88 : 126) which is unexplained. None of the Dichætes were dilute, which means that the gene for dilute is in the third-chromosome. But not all of the not-Dichætes were dilute, and this was attributed to the fact that the difference between the dilute and the wild-type was slight and fluctuation in dilute was observedly great. It is possible, however, that these peculiarities were of a different kind than supposed; but no further tests of the situation were made, since dilute was held to be a "poor" character. The stock was lost, but the vermilion forked stock still contains individuals that are probably the same dilute.

CURLED (cu).

(Figures 20*c*, 20*d*, page 152.)

ORIGIN OF CURLED.

The striking character "curled" wings was found by Morgan to be present in considerable numbers in a double-recessive stock of the second-chromosome mutants black and speck (December 15, 1915). Black speck curled flies were mated together and a pure stock was at once obtained.

DESCRIPTION OF CURLED.

The wings of the curled mutant are similar to those of jaunty, but turn up at the end more sharply and more extensively. The posterior third of the wing is bent upward, so that the tip is perpendicular or even curled forward. There is considerable fluctuation in the amount of curling, which is most pronounced in large young flies, and may be barely detectable in small flies that are from starved larvæ. The curled wings are usually slightly spread. In texture the wing is thin (like curved) and has a silvery or waxy appearance. The scutellum is small and pointed. The posterior scutellar bristles are erect as in vestigial, but cross more sharply, so that the tip of the left bristle points across the scutellum to the right, and *vice versa*. The body-color is dark, and the trident-pattern on the thorax is very prominent. The hairs and bristles and body-surface generally have the appearance of having been wet.

Because of the large fluctuation in the curliness it was found difficult to classify curled in the presence of Dichæte. More dependable characteristics of the mutant are the erect scutellar bristles and the waxy texture of the wings.

BLACK SPECK CURLED × WILD; CURLED NOT IN SECOND CHROMOSOME.

Since the curled mutation had been found in the black speck stock, a triple recessive was on hand by means of which a back-cross test could be made that would tell whether or not the gene for curled is in the second-chromosome with black and speck. A black speck curled male was out-crossed to a wild female, and three of the F_1 wild-type females were back-crossed to black speck curled males (table 106).

TABLE 106.—*P_1, black speck curled ♂ × wild ♀; B. C., F_1 + ♀ × b sp cu ♂.*

July 7, 1916.	b sp	b sp cu	+	cu	b	b cu	sp	sp cu
A.....	15	16	12	12	12	5	15	9
B.....	7	9	18	11	18	7	15	9
C.....	12	2	6	8	6	6	8	3
Total.....	34	27	36	31	36	18	38	21

The curled flies were slightly less numerous than those not-curled. They were distributed at random with respect to black and speck; hence it seemed unlikely that the gene for curled was in the second chromosome, though a position far to the right of black (i. e., near Star) was not excluded.

CURLED × DICHÆTE; CURLED IN III.

The view that the locus of curled is in the third-chromosome was tested by crossing curled to the third-chromosome dominant Dichæte, and back-crossing F_1 Dichæte females to curled males (table 107). The strong linkage

TABLE 107.—*P_1, curled ♂ × Dichæte ♀; B. C., F_1 + ♀ × cu ♂.*

July, 1916.	D	cu	D cu	+
A.....	120	207	7	4
B.....	123	78	4	9
Total.....	243	285	11	13

shown in the back-cross proved that the gene for curled is in the third chromosome. There were 24 recombinations in a total of 552 flies, or 4.3 per cent of recombination.

DICHÆTE CURLED × HAIRLESS.

In order to determine on which side of Dichæte curled is situated, it was necessary to know the linkage relations of curled with some other mapped locus besides Dichæte. The dominant "Hairless" had just been found and located approximately at ebony. This mutant was crossed to Dichæte curled from the preceding experiment, and the F_1 Dichæte Hairless females were crossed to curled males. The back-cross progeny showed that the locus of curled is between those of Dichæte and spineless; i. e., to the right of Dichæte.

SEPIA CURLED SPINELESS SOOTY ROUGH × DICHÆTE HAIRLESS.

The position of curled at approximately 4.3 to the right of Dichæte made curled valuable as an alternate to pink. That is, the behavior of the region between Dichæte and spineless could be followed by use of curled if it was desired to use some other eye-color that would conflict with the classification of pink. Thus, Gowen used curled in combination with sepia (J. E. Z., 1919, p. 242). He ran a sepia curled spineless sooty rough × Dichæte Hairless back-cross in order to observe the correlation between the crossing-over in different regions of the third chromosome. The Dichæte curled recombination per cent in Gowen's back-cross was 2.8; but there was a cross-over variation present, so that this value is too small for calculating the normal position of curled. The Dichæte spineless value was 7.4 instead of 16.2, which is the standard D ss value. If the Dichæte curled value was changed in the same ratio, the locus of curled would be at 5.6± to the right of Dichæte, or at 46±.

The region in which curled is thus located is one in which many mutant loci (pink, Deformed, maroon, dwarf, ski–III, warped, mahogany, compressed, scarlet, and varnished) are situated close to one another. The serial order of several of these is still unknown, and to locate curled with respect to them would require considerable work. This region seems peculiarly susceptible to cross-over variation, so that it is not sufficient to locate curled with reference to a point even as close as Dichæte. It is proposed to use pink or perhaps the dominant Deformed, as the base of reference of curled and the rest of this group.

TWO-BRISTLE (2b).

ORIGIN OF TWO-BRISTLE.

In determining whether the duplication that affected sable also affected the neighboring locus miniature, a sable-duplication sable garnet male was crossed to an eosine miniature female, and several F_2 cultures were raised. In one of these (8311, February 22, 1916) Bridges found a single male that had two bristles only of the normal four dorso-centrals. The mutation was similar to Dichæte in this respect, but the wings were normal.

DOMINANCE OF TWO-BRISTLE.

This "Two-bristle" male was out-crossed to yellow achæte females. Achæte is a sex-linked bristle modification of the same type as Two-bristle. The sons were all achæte, and about half of the daughters lacked one or more bristles from the thorax or the scutellum or both. It was judged that Two-bristles was not an allelomorph of achæte, since somewhat more than half of the daughters

were entirely wild-type (3625: +♀ 42, 2b ♀ 29, yac ♂ 37). The alternative is that Two-bristle is a dominant. Some further crosses, in the process of eliminating achæte, proved this view to be correct.

TWO-BRISTLE × STAR, MALE BACK-CROSS.

In order to test whether the gene for Two-bristle was in the second-chromosome, a Two-bristle male was out-crossed to Star (1286, April 21, 1916; S 14, 2b 6, S 2b 6, +6), and some of the F_1 Star Two-bristle males were out-crossed to wild females (table 108). The Two-bristle character appeared as often

TABLE 108.—*P_1, Two-bristle ♂ × Star ♀; B. C., F_1 S 2b ♂ × wild ♀.*

May 5, 1916.	S	2b	S 2b	+
4,470.....	48	32	26	65
4,471.....	44	27	33	84

among the Star as among the not-Star offspring, from which it follows that the gene for two-bristle is not a member of the second-chromosome group.

TWO-BRISTLE × SEPLE, MALE BACK-CROSS.

In testing the relation of Two-bristle to the third chromosome, the "seple" multiple was used (se ss e^s ro). In the F_1 from the cross of Two-bristle to seple a surer means of identification was used (4641, 2b 38, +44; 4644, 2b 27, +44). In the previous cultures the proportion of Two-bristle had always been less than the expected half, and it was thought that this was due to overlap of Two-bristle into the wild-type class. A careful examination was made of Two-bristle flies in order to see whether, as in the striking case of club, another more reliable somatic index of the mutant might not be present. Such an index was found in the invariable absence of the two small bristles that project backward from the top of the head just behind the ocelli (the postvertical bristles). When an F_1 Two-bristle male was back-crossed to a seple female, there were produced 45 Two-bristle and 68 seple individuals, with no recombination classes (4802, June 7, 1916). The locus of Two-bristle is therefore in the third chromosome.

TWO-BRISTLE × SEPLE, FEMALE BACK-CROSS.

The Star by Two-bristle result had made it probable that the locus of Two-bristle was in the third chromosome. Accordingly, a female back-cross (table 109, 4854) was carried out parallel to the male back-cross just described. Seple had been chosen for these crosses because the results from the female back-cross would show the location of Two-bristle within the third chromosome. Two-bristle came out in recombination with all the characters of seple in different degrees, there being only a single recombination representative for Two-bristle and spineless. The locus of Two-bristle is thus known to be very close to spineless, and to the left. But since the view that Two-bristle lies to the

left of spineless rested upon a single individual, more cultures of the same nature were raised. No other recombinations of Two-bristle and spineless were observed in the total of 1,176 flies (table 109).

TABLE 109.—*B. C., F_1 2b ♀* $\left(\frac{se}{2b}\ \frac{ss}{e^s}\ \frac{e^s}{}\ \frac{ro}{}\right) \times se\ ss\ e^s\ ro\ ♂$.

July 4, 1916.	se ss e^s ro	2b	se 2b ss e^s ro		se ss 2b e^s ro		se ss e^s	2b ro	se 2b e^s ro	ss	se 2b ro	ss e^s	2b ss e^s	2b e^s	ss ro
4,854	30	29	16	11	3	6	7	7	4	2	7	4	1	1	..
4,878	53	48	15	12	8	8	17	14	6	5	2	4	....	....	....
4,882	38	48	19	6	8	5	10	17	5	5	7	4	....	....	....
4,883	32	26	7	12	2	11	4	9	3	..	4	1	....	..	1
4,917	53	76	11	19	6	7	16	8	1	..	4	3	....	1	....
4,932	43	52	14	18	12	12	11	15	1	1	4	4	....	....	....
4,933	38	52	18	16	10	6	13	13	1	2	4	6	....	1	....
Total	287	331	100	94	49	55	78	83	21	15	32	26	1	3	1

TWO-BRISTLE SOOTY × SEPIA SPINELESS ROUGH, ALTERNATED BACK-CROSS.

Stocks of Two-bristle sooty and of sepia spineless rough were prepared to run an "alternated" back-cross, that is, one in which the mutant loci involved are equally divided between and alternate in position along the two chromosomes, thus, $\frac{se\ +\ ss\ +\ ro}{+\ 2b\ +\ e^s\ +}$. This type of back-cross is the best single test as regards viability disturbances, and is used frequently in spite of the

TABLE 110.—*Alternated B. C., F_1 2b ♀* $\left(\frac{se}{2b}\ \frac{ss}{e^s}\ \frac{ro}{}\right) \times se\ ss\ e^s\ ro\ ♂$.

Dec. 24, 1916.	se ss ro	2b e^s	se 2b e^s	ss ro	se e^s	se ss e^s	2b ro	se ss	2b e^s ro	se 2b ro	ss e^s	so 2b e^s ro	ss	2b ss	se ss e^s ro	2b	se 2b
6,251	82	71	20	27	.....	12	7	16	25	4	2	4	6	....	..	1	1
6,253	50	33	19	12	.....	7	8	10	12	1	3	3	5	1	1	.	.....
6,254	64	70	6	14	1	10	11	22	20	3	..	1	2	.....	1	1	.....
6,311	28	24	16	9	.....	5	7	14	10	4	2	7	7	.....	2	..	.....
Total	224	198	61	62	1	34	33	62	67	12	7	15	20	1	4	2	1

considerable labor in preparing the two alternating stocks and the multiple-recessive stock. The results of the alternated back-cross (table 110) were closely similar to those of the seple × Two-bristle cross. Two more recombinations for Two-bristle and spineless appeared in the total of 804 flies, and all three agreed in a position for Two-bristle to the left of spineless. Three recombinations in a total of 1,980 mean that the distance between Two-bristle

and spineless is about 0.2 unit. These two loci, Two-bristle and spineless, are practically to be treated as one base, the data involving Two-bristle being combined with those for spineless with a simple correction of 0.2 unit.

The mutant Two-bristle was not used further, and the stock was lost not long after the determination of the location. In carrying on the stock it became apparent that homozygous Two-bristle is lethal, as is the case with most other dominants in *Drosophila*.

BLACK-LEG.

In determining the chromosome of the sex-limited character "bobbed," Bridges raised several F_2 cultures from the cross of bobbed to black pink bent. Bobbed was shown to be sex-linked as well as limited in expression entirely to the female. In one of the F_2 cultures (3344, February 23, 1916) it was observed that many of the flies were pale in body-color, "pallid," with wings that were thin-textured and yellowish and either arched or slightly crumpled, and with hind-legs that were bowed and possessed unusually dark coloration of the tibial joint.

These "black-legs" were almost entirely pink-eyed, from which it followed that the locus of the recessive mutant is in the third chromosome not far from that of pink.

The black-leg females were generally sterile and very unproductive, and some trouble was met with in the use of males also.

A pink black-leg back-cross gave 21 pink black-leg and 38 wild-type flies with no recombinations (4413, April 27, 1916). However, in keeping stock of black-legs a few recombinations were observed.

The classification of the character was not sharp, and the handling of the stock was so difficult that the mutant was discarded without further tests. It is still present in the black pink bent stock, as some crosses in 1921 showed.

EXTRA-SCUTELLARS–III.

In Indiana University Study No. 36 (March 1918), Fernandus Payne reports the results of selection for an increased number of bristles on the scutellum.

Early in 1916, a female with one extra bristle was mated to a wild-type brother, and there were produced 226 wild-type flies and 2 females that had an extra bristle. These two females produced about 4 per cent of extra-bristle offspring, including a few with two extras. In the next generation the percentage of extra-bristle flies rose to 11.5, a few flies having three extras. The selection was carried on for 38 generations, using as parents each generation flies that were among the extremes available. After the twenty-ninth generation the results were uniformly the same—all the flies had extra bristles, the mode being at 5 extras, and the extreme being 11 extras.

This level was attained through a series of rises separated by periods of uniformity. The beginning of each rise represents mutation, or the attainment of a particular aggregate of genes through assortment or crossing-over. The rise continues until all the parents used are of the new constitution. The succeeding period of uniformity lasts until a new change in constitution is initiated. An examination of the data shows four distinct levels for the

mode, each preceded by a sharp rise. The breaks occurred at the fourth, the eighth, the fifteenth, and the twenty-ninth generations. The percentages of flies with extra bristles showed similar breaks at these generations, especially marked at the eighth and fifteenth. The appearance of the original 5-bristled female represents another break; and the rapid rise during the early generations of selection probably represent two or three breaks merged together. The course of the curve represents four to six or more selected constitutions and an unknown number of discarded constitutions.

GENETICAL ANALYSIS OF THE RESULT.

Out-crosses of flies from the high line showed that at least one of the genes involved was an autosomal dominant and at least one was sex-linked.

An extra-bristle female was out-crossed to eosin ruby forked, and an F_1 extra-bristle female again out-crossed to eosin ruby forked male. The results showed that there was a dominant gene located in the X chromosome near eosin, probably at about a third of the distance from eosin to ruby.

An extra-bristled female was out-crossed to a black pink bent male, and F_1 extra-bristled flies inbred for F_2. The distribution of extra bristles among the black flies was the same as among the not-blacks, which showed that there was no second-chromosome mutant involved, unless it were a dominant at one or the other end of the chromosome. The percentage of extras among the bents was even slightly higher than among the not-bents, showing that the fourth-chromosome of the selected line was not more positive than the fourth-chromosome carrying bent. But only 16 per cent of the pink flies showed extra-bristles, while 67 per cent of the not-pink flies showed extras. This demonstrates that at least one gene for extra bristles was carried by the third-chromosome of the selected line.

Other crosses with third-chromosome mutants confirmed the presence of a third-chromosome modifier. Other crosses with second-chromosome mutants, and with the fourth-chromosome mutant eyeless, showed that there was probably no second- and no fourth-chromosome modifiers at work.

The localization of the third-chromosome modifier within the third chromosome was attempted, by raising F_2 from the cross of extras to seple. The attempt was only partly successful, because of the presence of a dominant cross-over modifier (Chip, see p. 183) in the third chromosome of the extra-bristle stock. None of the 8 ss e^s ro flies had extra-bristles, while two of the 7 se flies had extra-bristles. Since crossing-over between sepia and spineless transferred the modification to sepia, the locus of the modifier is to the right of sepia, probably to the right of the mid-point of the chromosome, i. e., pink.

SCUTE-INHIBITOR-III.

In the sixth generation of Payne's selection for increased number of scutellar bristles (see p. 158) appeared a single male having only 1 bristle on the scutellum instead of the normal 4 (Fernandus Payne, Genetics, 5: 501–542, November 1920). Breeding tests showed that the character is a sex-linked recessive situated to the left of eosin. The position and description of "reduced" tallies with that of "scute," with which it is probably identical. Payne started selec-

tion within the scute strain for a more intensified, and for a less intensified reduction of the scutellar bristles. Both selections were successful.

SCUTE SELECTED TOWARD THE WILD-TYPE.

The selection for a less marked departure from the wild-type resulted in a strain in which about half the flies were wild-type as far as scutellar bristles were concerned, though they could still be distinguished from wild-type by the absence of ocellar and post-vertical bristles. The mean of this line differed from the wild-type by only about half a bristle.

Linkage tests showed that there was present in the X-chromosome of this slight stock a modifier gene near miniature that tended to inhibit the reduction of bristles. A cross to black pink bent, with back-cross test of the F_1 wild-type female by black pink bent males showed that there was no dominant inhibitor of reduction in the fourth-chromosome, but that there was one in the third- and another in the second-chromosome. This test could detect only dominant modifiers. The presence of a dominant third-chromosome gene tending to reverse the action of the scute gene was confirmed by similar back-crosses with spineless and sooty. A comparison of the results of the back-crosses with pink, with spineless, and with sooty showed that the locus of the modifier was closest to spineless and farthest from pink (ss with scutellar-bristles = 6 per cent; e^s with = 18 per cent; pink with = 35 per cent). This position was confirmed by finding that the dominant was still farther removed from sepia (40 per cent of se with scutellar) and farthest of all from rough (ro with = 48 per cent). The agreement of these findings with a position very close to spineless probably means that there was only one dominant in the third chromosome.

Since scute arose in the line selected for increased number of scutellar bristles, it seems likely that this dominant is the same as that found in the analysis reported on page 158. This *a priori* probability was strengthened by finding that when the scute flies that had an abnormally large number of bristles (3.5 on the average) were out-crossed to wild the F_1 flies also had an abnormally large number of bristles. That is, the modifier that raises the number of bristles of scute also raises the number of bristles of the not-scute, as the previously mentioned modifier was known to do. The locus of the present modifier agrees with that in the previous experiment, for the cross of extra-bristles to seple had shown that the locus of the modifier was to the right of sepia and probably to the right of the mid-point of the chromosome (i. e., pink). From the identity of origin, and similarity in effects, in dominance, and in location Payne concluded that the scute-inhibitor in his selection for higher bristled scute was the same gene as that present in his selection for extra bristles.

SCUTE SELECTED AWAY FROM THE WILD-TYPE.

The selection for a more marked reduction of scutellar bristles resulted in a line in which about 98 per cent of the flies had no scutellar bristles at all. Tests of this line showed that it lacked the sex-linked modifier near miniature that was present in the scute line selected in the opposite direction and also lacked the second-chromosome dominant and the third-chromosome dominant inhibitors of scute. The selection had eliminated three modifiers (at least)

that had tended to make the number of bristles on the scutellum larger. That the selection possibly incorporated a dominant tending to reduce scutellar bristles is suggested by the results of the back-cross with black pink bent. In the offspring from the second out-cross to black pink bent (back-cross offspring) only 93 per cent of the scute flies had no scutellars, while in the original stock 98 per cent lacked scutellars. Among the black flies the percentage without scutellars was 97, among the bent 95, and among the pink 91. That is, a dominant third-chromosome intensifier of scute may have been present in the stock selected for fewer scutellar bristles. The difference is not great enough to furnish more than an indication of such an intensifier.

HAIRLESS (II).

(Figure 21.)

ORIGIN OF HAIRLESS.

In the F_2 of a cross of pink spineless to a new recessive mutant "telescope," that proved to be in the second chromosome, Bridges found a single pink

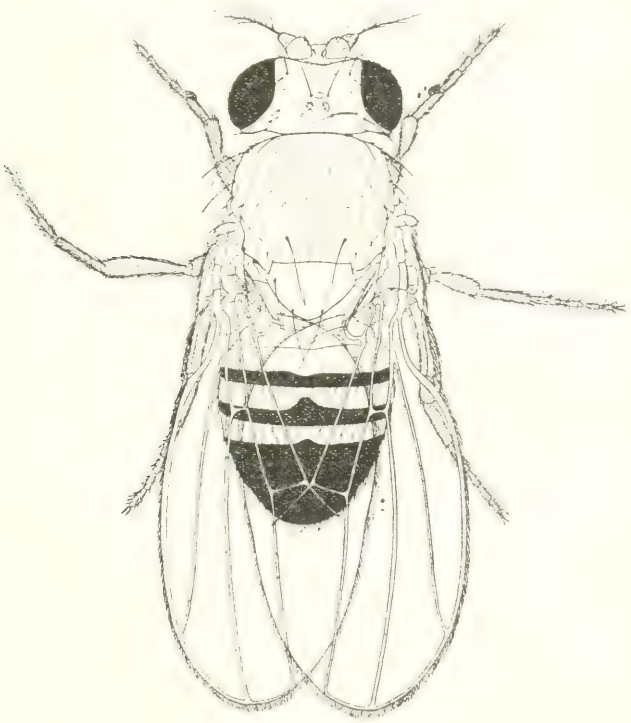


FIG. 21.—Hairless.

spineless male from which several bristles and also large areas of the hairs (microchætæ) on the dorsal surface of the thorax were absent (culture 3503, March 4, 1916).

DOMINANCE OF HAIRLESS; DESCRIPTION OF HAIRLESS.

The pink spineless "Hairless" male was out-crossed to the third-chromosome dominant Dichæte, and the gene was found to be dominant (table 111). The Hairless character did not appear as such in the F_1 flies; but instead, these flies showed a character very similar to Two-bristle, which had been found just previously. The F_1 Hairless flies lacked the two post-vertical bristles that ordinarily project backwards from the head in the region of the ocelli. They also lacked one or both of the anterior dorso-centrals, and probably a majority of the bristles that project backward from the posterior edge of each sclerite of the abdomen. There remained to mark the site of each of these absent bristles the basal ring in which the bristle is normally mounted. Apparently the bristles have weak attachments and break off during emergence from the pupa case. In the cases of Two-bristle, Dichæte and the other mutants in which bristles are absent, this basal ring also is absent; in those mutants the bristle is never formed. Recently (November 1920) a fourth-chromosome recessive "shaven" has been found, in which both bristles and hairs are absent in varying degrees from the entire fly, but especially from the abdomen; but the basal rings are present, and certain grades of shaven bear a startling resemblance to Hairless.

There is one further characteristic of Hairless that is very useful in classification, and which is not possessed by shaven, viz., a break in the fourth and fifth longitudinal veins. In consequence they fail to reach the margin of the wing. There is considerable fluctuation in the number of bristles that are absent, the most useful index being the post-verticals. No individuals have been seen in which the character was not apparent in the abdominal bristles, although one very rarely need resort to examination of these bristles, which are inconveniently small and are covered by the wings. Recently Sturtevant has made a careful examination of the bristles of Hairless, chiefly with respect to the thorax and scutellum.

PINK SPINELESS HAIRLESS × DICHÆTE, MALE BACK-CROSS.

An F_1 Dichæte Hairless male from table 111 was back-crossed to a pink spineless female, and there were produced 63 D and 62 p ss H flies with no recombinations (4183, April 10, 1916). This male back-cross result proved that the locus of Hairless is in the third-chromosome.

TABLE 111.—*P_1, pink spineless Hairless ♂ × Dichæte ♀.*

March 9, 1916.	D	H	D H	+
3,850.....	98	100	91	91

DICHÆTE × HAIRLESS, FEMALE BACK-CROSS.

Two F_1 Dichæte Hairless females were out-crossed to wild males, with the production of 26.4 per cent of recombination (table 112).

TABLE 112.—*B. C., F_1 Dichæte Hairless ♀ (3850) × wild ♂.*

April 6, 1916.	D	H	D H	+
4,133	51	52	11	17
4,134	47	64	22	27
Total	98	116	33	44

DICHÆTE × PINK SPINELESS HAIRLESS, FEMALE BACK-CROSS.

The results of table 112 showed that the loci of Dichæte and Hairless were 29 units apart (26.4+2.6± of correction for doubles), but did not indicate whether Hairless lies to the left or right. A Dichæte × pink spineless Hairless female back-cross (table 113) showed that Hairless lies to the right, and

TABLE 113.—*P_1, pink spineless Hairless ♂ × Dichæte ♀; B. C., F_1 D H ♀ × p ss ♂.*

June 21, 1916.	D	p ss H	D p ss H	+	D ss H	p	D H	p ss	ss H	D p ss
4,627	44	41	4	4	8	5	6	4	1	.....
4,628	53	40	3	5	6	2	9	6	.....	2
Total	97	81	7	9	14	7	15	10	1	2

further, that it is some 11 units to the right of spineless. This last fact placed the locus of Hairless very close to that of ebony.

MODIFICATION OF HAIRLESS BY SPINELESS.

In the back-crosses in which both Hairless and spineless were involved, the spineless Hairless individuals were of the original type, that is, they lacked certain hairs as well as the bristles. Furthermore, the extent of the terminal gap in the fourth- and fifth-longitudinal veins was much increased, so that these veins apparently never reached the margin in such flies. The classification of these two characters into the four combinations is particularly easy.

The character Hairless is also exaggerated in the absence of one of the fourth chromosomes (Bridges, 1921).

Further experiments have shown (table 2) that the locus of Hairless is about 1.2 units to the left of that of ebony. Most of the remaining data is

recorded in connection with other mutants, two small experiments having no other connection (tables 114 and 115).

TABLE 114.—*B. C., F_1 Dichæte Hairless* ♀ (*table* —) × *wild* ♂.

March 8, 1918.	D	H	D H	+
8,507	109	120	39	41
8,510	127	138	46	42
Total	236	258	85	83

TABLE 115.—*P_1, Dichæte sooty* ♂ × *Hairless* ♀; *B. C., F_1 D H* ♀ × e^s ♂.

March 9, 1918.	D e^s	H	D H	e^s	D	H e^s
8,509	122	129	35	35	6	3

LETHAL NATURE OF HOMOZYGOUS HAIRLESS.

As is the case with the third-chromosome dominants Beaded, Dichæte, Two-bristle, Delta, various Minutes, and with most of the second-chromosome dominants, Hairless is lethal when homozygous. This became apparent in the early attempts to obtain pure-breeding stock; but has since been definitely proved, first by F_2 results of Hairless sooty × wild, in which only a few sooty flies (resulting from crossing-over) emerged, and finally by breeding a homogeneous balanced stock in which CIII and IIIIa were in one chromosome, Hairless in the other. This stock maintains its heterozygous constitution permanently by the death of both homozygous types.

EVALUATION OF HAIRLESS.

Hairless and ebony are situated so close together that they are treated as a single base, which is second only to Dichæte in importance; both Hairless and ebony are in themselves mutants of first rank. Hairless has normal viability, fertility and productivity. There is considerable fluctuation in the Hairless character, and the classification is not rapid, but the separations are accurate and complete. Hairless is usable without confusion with hairy, Dichæte, spineless, and the various Minutes, and with all the mutations not affecting bristles. A double-dominant stock Dichæte Hairless was made up, which is now the standard stock for use in the preliminary location of third-chromosome mutants, corresponding to the eosin vermilion forked stock for the X-chromosome. The most-used stock in our laboratory is Star Dichæte; but in cases in which another winged mutant is concerned, a Star Hairless stock is used instead.

PINK[3] (p^3).

In locating sex-linked lethal-11 by means of a cross to the recessives eosin vermilion small-wing forked, Bridges found that about a quarter of the off-

spring of culture 4291 had a light pink eye-color (April 21, 1916). This eye-color was lighter than true "pink" and not so yellowish as "peach"; it was crossed to pink and found to give an intermediate compound. At the same time it was crossed to Dichæte, and a male back-cross gave proof of its third-chromosome location. The mutation, the third allelomorph of pink, was lost almost immediately.

LETHAL–IIIE (1264, A. H. S.).

In selecting for a higher number of bristles, Sturtevant found that one culture of Dichæte by Dichæte produced 60 Dichæte and no not-Dichæte offspring, instead of the usual 2 Dichæte to 1 not-Dichæte ratio (1246, A. H. S., May, 1916). This line was continued for about 18 generations through continued Dichæte by Dichæte mating, and it produced in total 2,735 Dichætes and 4 not-Dichætes. The production of the 4 not-Dichætes suggested that the Dichætes were really all heterozygous, but that the stock was balanced in a manner already known for Truncate and for Beaded. That is, in the not-Dichæte chromosome a recessive lethal was present in a locus close to that of Dichæte, and both the homozygous lethal and the homozygous Dichæte flies died, leaving only the double heterozygous types to be counted. The four not-Dichæte flies were due to crossing-over between Dichæte and the lethal, so that an egg carrying neither Dichæte nor the lethal was produced. That the Dichætes of this nearly pure-breeding strain were actually heterozygous was shown by out-crossing them; half the offspring were Dichæte and half not. That a lethal was present in the not-Dichæte chromosome was proved by outcrossing the Dichæte and then breeding from the not-Dichæte F_1 flies. These were shown to carry a lethal. This lethal (IIIIe) is still on hand, being used to balance the stock of the Extended mutant in the same way in which the Dichæte line was balanced.

LOCUS OF THE LETHAL.

By studying the constitution of the 4 not-Dichæte flies, it was found that the locus of the lethal is to the right of that of Dichæte. The Dichæte strain was carrying other third-chromosome mutants as well as Dichæte, and the test for relative order was to find which of these mutants had been gained by the crossing-over that removed the lethal.

The distance between Dichæte and the lethal is easily determined, since the not-Dichætes are a simple class by recombination ($rc=4$), and the Dichætes are a complex class, mainly original combinations ($2oc+1rc=2735$). From these two equations the recombination per cent is 0.3, which is also the distance in units.

EXTENDED (D^E).

(Figure 22*b*.)

In the course of a selection experiment, Dichæte flies were mated together in pairs (Sturtevant, 1918). On June 11, 1916, it was noticed that one of these pairs (culture 1379) was giving numerous flies intermediate in appearance between Dichæte and wild-type. They had the bristles of the wild-type,

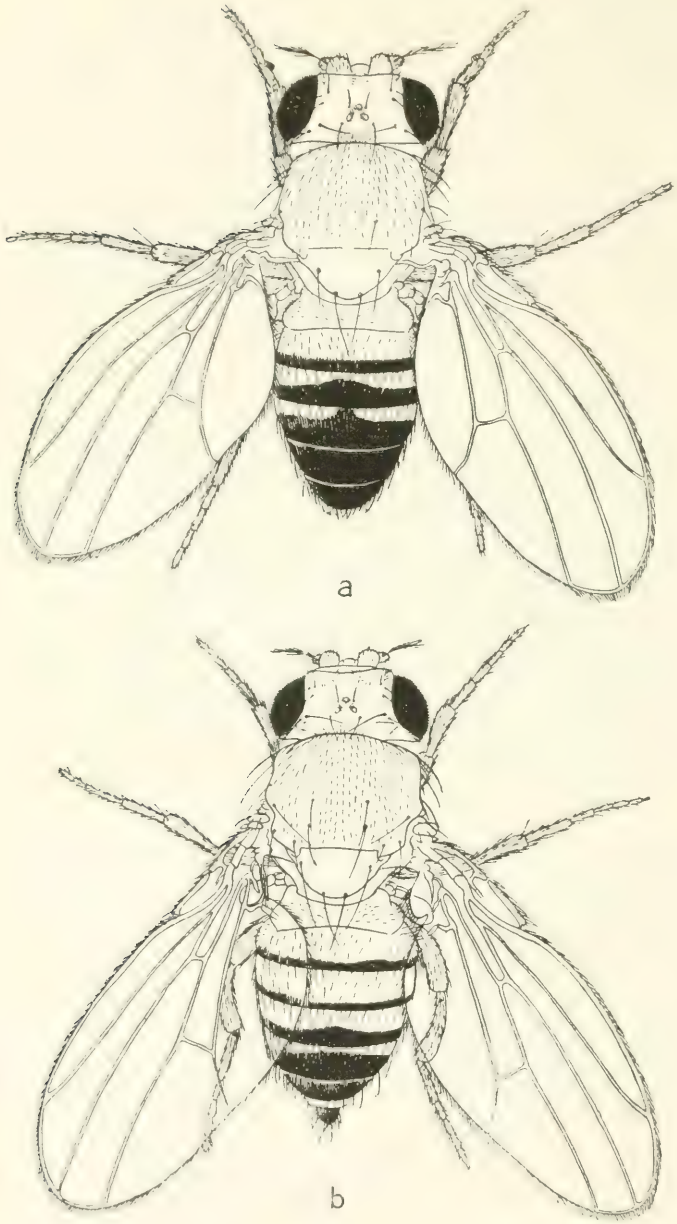


FIG. 22.—Dichæte (a), and its allelomorph Extended (b).

but the wings were more or less extended like those of Dichæte. These "Extended" flies were shown to carry an allelomorph of Dichæte that is dominant lethal, and lethal with Dichæte (i. e., the compounds die). The character Extended overlaps normal sufficiently to make it unsatisfactory for studies of linkage, but is distinct enough so that its allelomorphism to Dichæte could be established. The counts obtained by Sturtevant (1918, pp. 31, 32) give sepia Extended $= \frac{13 \times 100}{105} = 12.4$. Extended spineless $= \frac{11 \times 100}{144} = 7.6$.

One culture, in which all three loci (se D^{E} ss) were followed, showed the sequence to be sepia, Extended, spineless. The Extended locus was thus shown to be not far from that of Dichæte. That the gene was lethal was shown by the failure of the attempts to get homozygous Extendeds. That Dichæte Extended compounds die was shown by mating Dichæte to Extended. The F_1 result was, Dichæte 99, Extended 69, wild-type 102. The ratio expected if the compounds die is 1:1:1, and this is approximated if we remember that some Extendeds were undoubtedly classified as wild-type. The deficit of Dichæte (one-third the total instead of one-half) and the facts that F_1 Dichætes failed to produce Extendeds and F_1 Extendeds failed to produce Dichætes, constitute a demonstration that Dichæte-Extended flies die.

Sturtevant (1918, pp. 32, 33) has shown that the Extended mutation arose in a Dichæte fly that had been selected for extra bristles. It is not possible to determine whether the mutation arose in the Dichæte gene of that fly or in the normal allelomorph. Sturtevant has presented arguments against supposing the gene to have arisen by "contamination" of the two allelomorphs, and against supposing that the plus selection had anything to do with the *production* of the mutant. It is evident from his account that the *discovery* of the mutant was due to the selection that was carried out; but that point does not bear on the question as to the cause of the initial germinal change.

LETHAL–IIIF (lIIIf).

In the same line in which a previous lethal (lIIIe) had been found (see p. 165), viz., a line maintained by continually inbreeding Dichæte flies having the highest bristle-number, a second lethal (lIIIf) appeared (1546, A. H. S., June 30, 1916). The not-Dichæte chromosome was carrying sepia, spineless, sooty, and rough ("seple"). There were produced 154 Dichæte (of various classes) and one not-Dichæte that was spineless, sooty, rough. A crossing-over had evidently removed the lethal (and sepia) from seple without inserting the Dichæte gene. The locus of the lethal is therefore to the left of that of Dichæte. The distance between the lethal and Dichæte is 1.3 units (oc=1; 2oc+1rc=154). The fact that this second lethal is situated to the left of Dichæte makes it certain that it is a new lethal. The non-allelomorphic nature of the lethals was proved by crossing the two lines, both of which were practically pure-breeding for the Dichæte character. The cross gave the ordinary 2D:1 not-Dichæte results, from which it is apparent that flies heterozygous for both lethals live. The stock of this second lethal was discarded at once.

VORTEX–III (VOIII).

(Figure 23.)

ORIGIN OF VORTEX.

In a wild stock, that had been caught in California, Bridges found (August 7, 1916) some flies with a pair of whorls of bristles on the thorax. At the center of each vortex was a pit, or sometimes an elevation like a volcanic cone and crater. There was considerable variation in the development of this "vortex"

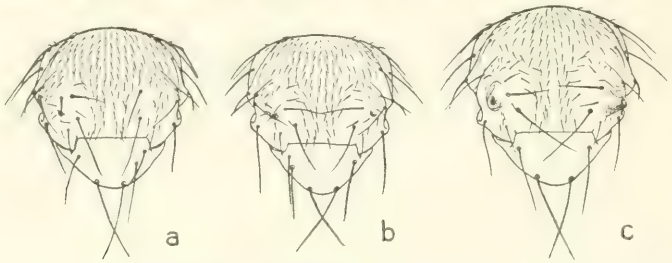


FIG. 23.—Vortex. (a) extreme vortex; (b) average vortex; (c) slight vortex.

character, but a stock was secured in which all the individuals showed the character to some degree. In the late hatches of a stock culture, the grade of the vortex character became gradually less extreme, and some of the males became indistinguishable somatically from wild-type.

VORTEX × STAR DICHÆTE.

A vortex female was crossed to a Star Dichæte male, and F_1 Star Dichæte males were mated to vortex females. A full account of this and subsequent crosses is to be found in a paper by Bridges and Mohr in Genetics, May 1919, pp. 283-306. For the present account it is sufficient to summarize the results by stating that the character vortex was found to depend upon a second-chromosome recessive and a third-chromosome recessive, neither of which produces any somatic effect by itself.

The vortex character never appears unless the fly is homozygous for vortex–II, and at least heterozygous for vortex–III. Only a small proportion of the homozygous vortex–II, heterozygous vortex–III flies, and these only when females (about 15 per cent of such females) showed the vortex character. Flies homozygous for vortex–III, and heterozygous for vortex II were invariably wild-type.

THE LOCUS OF VORTEX-III.

Female back-crosses of Star Dichæte to vortex showed no crossing-over between Dichæte and vortex–III. A second linkage experiment of vortex–III × Dichæte Hairless was carried out, and here too there were no recombinations for Dichæte and vortex–III. Vortex–III is not allelomorphic to Dichæte, but is situated very close to Dichæte.

AN INTENSIFIER OF THE DOMINANCE OF VORTEX III.

In some of the early work, cultures were found in which a higher proportion (50% or more) of the females that were homozygous for vortex–II and

heterozygous for vortex–III showed the vortex character. This same condition reappeared, and was found to be due to a dominant intensifier of vortex situated probably in the second-chromosome. In homozygous condition this intensifier even made a small proportion of the heterozygous–III males show the vortex character.

VORTEX-III NOT A CROSSING-OVER SUPPRESSOR.

The vortex–III × Dichæte back-crosses had given no flies representing crossing-over between these two loci in a total of 739 flies. This was taken to mean that these two loci are very close together; but another interpretation was possible, viz, that vortex–III itself acted as a suppressor of crossing-over for the region between the two loci, or that such a suppressor, of independent origin by mutation, was present with vortex–III. To test this question, vortex

TABLE 116.—P_1, *vortex × roughoid hairy scarlet peach spineless sooty; B. C., F_1 + ♀ × "III-ple" ♂.*

April 29, 1922	ru h st p^p ss e^s +	ru h st p^p ss e^s	ru h st p^p ss e^s	ru h st p^p ss e^s	ru h st p^p ss e^s	ru h st p^p ss e^s	ru h st p^p ss e^s	ru h st p^p ss e^s	ru h st p^p ss e^s	ru h st p^p ss e^s	ru h st p^p ss e^s	ru h st p^p ss e^s	ru h st p^p ss e^s	ru h st p^p ss e^s	ru h st p^p ss e^s	ru h st p^p ss e^s	ru h st p^p ss e^s	ru h st p^p ss e^s
13,130...	94 114	52 25	39 29	5 3	9 12	17 17	5 1	2 2	2 5	2 2	2	1 6	3 1	1	2 1	.	1	...
13,131...	107 103	18 35	34 23	3 1	15 8	22 24	2 1	.....	1 4	5 2	...	1 3	5 6	...	1 ..	1	...	1
Total..	201 217	70 60	73 52	8 4	24 20	39 41	7 2	2 2	3 9	7 4	2	2 9	8 7	1	3 1	1	1	1

was crossed to the multiple-recessive III–ple (roughoid hairy scarlet peach spineless sooty), and two F_1 wild-type females were mated to III–ple males (table 116). The back-cross progeny did not differ greatly from standard in any particular. From this it follows that the loci of Dichæte and vortex–III are really very close together.

CLUBOID.

ORIGIN AND DESCRIPTION OF CLUBOID.

In observing the effect upon output of flies by the addition of various percentages of alcohol to the fermented banana culture-media, Bridges found that a mutant resembling club was present in the wild stock used (September 15, 1916). The wings of the "cluboid" mutant remained in the pupal condition or were usually expanded to some degree, as in the case of the old club. Quite often the wings were nearly fully expanded, but even in that case most of the cluboid flies could be detected by the small body-size and the glazed appearance of the body-surface. A stock of cluboid was obtained by breeding together males and females of the mutant. The females were of low productivity and often sterile.

CLUBOID × STAR DICHÆTE.

A cluboid male from the stock was out-crossed to Star Dichæte, and an F_1 Star Dichæte male was back-crossed to a cluboid female (table 117). None of the Dichæte offspring of the back-cross were cluboid, while about half of the

TABLE 117.—*P_1, cluboid × Star Dichæte; B. C., F_1 S D ♂ × cbd. ♀.*

Nov. 15, 1916.	cbd.	S cbd.	D	SD
5,843.....	25	25	27	46
B. C., F_1 S D ♀ × cbd. ♂.				
Nov. 16, 1916.	D	cbd.	D cbd.	+
5,864.....	59	44	22	18

Star offspring were, from which it is clear that the mutant is in the third-chromosome.

An F_1 Star Dichæte female was back-crossed to a cluboid male, and (Star disregarded) there was 28.0 per cent of recombination for Dichæte and cluboid. It is not known from this experiment on which side of Dichæte cluboid lies, although at the time the experiment was done it was assumed that the locus was to the right, since sepia was supposed to be at the left end. The stock of cluboid was lost before any further determination could be made.

DICHÆTE PLUS-MODIFIER.

That the bristle-number of Dichæte flies was subject to considerable variation was known, and Sturtevant carried out selections for both greater and lesser number of thoracic and scutellar bristles (Sturtevant, 1918). Both of these selections shifted the mean significantly. Experiments were then carried out to determine what was responsible for these differences. A Dichæte rough male from a high line (1002, A. H. S.) was crossed to a female from an "inbred speck" stock. F_1 females were then out-crossed to rough males. In the resulting offspring it was observed that the Dichæte rough flies had a significantly higher bristle-number than the Dichætes that had lost rough by crossing-over. The same cross-over that removed rough removed a dominant Plus-modifier located in the region near rough (Culture 2333, A. H. S.), September, 1916). No closer approximation of the locus of this modifier was made, and the stock was soon discarded.

ASCUTE (as).

ORIGIN AND DESCRIPTION OF ASCUTE.

In preparing a multiple stock to determine the position of bithorax more closely, sepia was crossed to Dichæte and an F_1 Dichæte female crossed to

seple again. In the back-cross progeny, Bridges found that half of the seple flies possessed a new mutant character (5597, October 21, 1916). This character involved the nearly complete obliteration of the furrow that normally divides the scutellum from the thorax; the anterior part of the scutellum is raised so as to be practically continuous with the thorax. This elevated anterior part may contain an air-bubble, from which it may be inferred that the alteration is primarily of the superficial layers. Occasionally there is a black spot, like a dried exudation, at a particular point on one or both sides of the scutellum.

DICHÆTE × ASCUTE ROUGH.

Approximately a quarter of the flies of the culture in which "ascute" was originally observed were ascute, and these flies were nearly all of the not-Dichæte class. Evidently the gene for ascute is in the third chromosome. The P_1 seple fly had been heterozygous for the ascute gene, and so had the seple male used in back-crossing. In making the counts attention was paid to only three characters, Dichæte, ascute and rough, since it was thought that Dichæte and rough would give a satisfactory preliminary determination of the ascute locus (table 118). Only one of the 43 ascute flies was at the same time Dichæte, so

TABLE 118.—*P_1, seple × Dichæte; B. C., F_1 D ♀ × seple ♂.*

Oct. 21, 1916.	as ro	D as ro	as	D	ro	D ro	+
5,597.....	21	1	21	62	37	35	21

that the locus of ascute is only about 2.3 units from that of Dichæte. This fly was at the same time rough, so that the locus is probably to the right of that of Dichæte.

DICHÆTE × SEPIA ASCUTE.

Rough is so far removed from Dichæte and ascute that it was not well suited for finding the location of ascute. A second culture was raised in which the father was an "ascute seple," and in which sepia, Dichæte, and ascute were

TABLE 119.—*P_1, "ascute seple" × Dichæte; B. C., F_1 D ♀ × "ascute seple" ♂.*

Nov. 15, 1916.	se as	D	se D	as	se	D as
5,831.....	174	215	24	22	2	3

followed (table 119). In this culture, five recombinations for Dichæte and ascute occurred, which indicates a position for ascute 1.1 units to the right of Dichæte.

"ASCUTE SEPLE" × DICHÆTE.

Three cultures were raised in which all the mutants of an "ascute seple" × Dichæte female back-cross were followed (table 120). These furnished 15 Dichæte ascute recombinations, corresponding to 3.3 units of distance between

TABLE 120.—*P_1, "ascute seple" × Dichæte; B. C., F_1 D ♀ × "ascute seple" ♂.*

Nov. 15, 1916.	se as ss e^s ro	D	se D as ss e^s ro	se D as ss e^s ro	se as D ss e^s ro	se as ss e^s D ro	se as ss e^s ro	se as D ss e^s ro	se as D ss ro e^s	D ss	se D as ss ro e^s
5											
6,828.....	38	59	10 3	2 .	5 5	8 11	18 16	. .	. 1	. 1	.	1 2
6,005.....	11	48	2 1	2 1	4 2	4 7	5 10	1 .	4 .	. .	1	. .
,035.....	30	60	3 4	6 4	9 3	8 7	14 17	1 1	. 1	1 .	.	1 .
Total...	79	167	15 8	10 5	18 10	20 25	37 43	2 1	4 2	1 1	1	2 2

Dichæte and ascute. The mean recombination per cent for Dichæte ascute on the basis of all the data is 2.2 units. But since the D ss and other values are lower than standard, the value 2.2 should be corrected. The most probable position for ascute is about 3 units to the right of Dichæte, or at 43.5.

ASCUTES 2, 3, AND 4.

The stock of ascute was discarded because of the poor viability of ascute that was apparent. (See table 120.) A new appearance of the ascute mutation, apparently identical with the original character, occurred in the Dichæte stock (December 16, 1918). This ascute character was with Dichæte, and kept reoccurring as a small proportion of the Dichæte flies of the stock. These Dichæte ascutes were due to crossing-over, and from them a new ascute stock was obtained. The small number of Dichæte ascutes shows that the locus of ascute is close to that of Dichæte, as is the known case with the original ascute. The bubble and the black spots were present in the new as in the old ascute. That it is an entirely new mutation in origin is certain from its association with Dichæte, while the old ascute was embedded in seple.

In experiments to locate the sex-linked recessive prune, Bridges found scute a third time, but there is nothing to prove that this ascute was the same as the previous two in location, or, on the other hand, that it might not have come from crosses with the Dichæte stock on some previous unknown occasion. No experiments were carried out with ascute–3.

In the kidney stock a fourth ascute was found that appeared the same somatically as the previous ones, and is certainly of separate origin, but whether allelomorphic or not is unknown.

SCARLET (st).

(Plate 1, Figure 11.)

ORIGIN OF SCARLET.

In a wild stock that had been bred at a high temperature, Mildred Hoge Richards found an eye-color mutation that was a brilliant vermilion, and

which was named scarlet (November 18, 1916). Stock was secured, and tests showed that scarlet was a simple autosomal recessive. (For a full account of scarlet see Biol. Bull. 36, 199–206, 1918.)

DESCRIPTION OF SCARLET.

In eye-color the scarlet mutation is a bright red or vermilion color that resembles sex-linked "vermilion" very closely, becoming darker with age as does "vermilion." But scarlet darkens sooner, and approaches the wild-type more closely in the final stage of darkening. Aside from this slight difference, the two are inseparable in appearance. The double recessive vermilion scarlet is likewise vermilion in color and indistinguishable from both single recessives. The ocellar color of scarlet is white, and this point enables one to classify even very old scarlets with accuracy, and to use scarlet in combination with sepia.

SCARLET × SOOTY; SCARLET × DICHÆTE.

It was found that scarlet gave independent assortment with blood (a white allelomorph, chromosome–I), with vestigial (chromosome–II), and with eyeless (chromosome–IV); but with sooty (chromosome–III) scarlet gave a typical 2 : 1 : 1 : 0 linkage ratio. A double recessive, scarlet sooty, was obtained from further breeding in this line; and a scarlet sooty back-cross test was carried out, that gave 1,318 recombinations in a total of 4,059 flies, or 32.4 per cent. At the same time, a Dichæte × scarlet back-cross was made, which gave 68 recombinations in a total of 1,801 flies, or 3.8 per cent. Dichæte and sooty were supposed to give about 20 per cent of recombination, and it seemed probable that the locus of scarlet was 3 units to the left of that of Dichæte, for the scarlet sooty value of 32 was considerably greater than the 17 expected if scarlet were to the right of Dichæte.

SCARLET BY REMUTATION.

The mutant scarlet was found independently as a remutation by D. E. Lancefield (March 18, 1918) in the F_2 from a cross of eosin miniature to reverted-Bar (for full account see Biol. Bull. 35, 207–210, October 1918). In this F_2 there were present, besides the expected eosin, a bright-red color similar to the sex-linked recessive vermilion, and a pink color that was combined with a wing modification. The "pink-wing" mutant proved to be a second-chromosome recessive, which has now been located at about 8 units to the right of black. The "vermilion" was proved, by a male back-cross with Star Dichæte, to be a third-chromosome recessive.

SCARLET × DICHÆTE; SCARLET × HAIRLESS.

Lancefield found that a back-cross of "vermilion" to Dichæte gave 15 recombinations in a total of 549 flies, or 2.7 per cent. Likewise, a back-cross with Hairless gave 140 recombinations in a total of 592 flies, or 25.4 per cent. At that time it was thought that Dichæte and Hairless gave about 19 per cent of recombination; but that value was based largely on the data of Gowen, which have now been shown to differ significantly from the remainder of the data on these loci. Consequently the locus of "vermilion" was supposed to be to the left of Dichæte by about 3 units.

The characteristics of scarlet, and its locus, determined by Mrs. Richards, agreed so closely with those for "vermilion" that this latter stock was sent to her for comparison. Mrs. Richards crossed the two stocks and found that the F_1 flies were all likewise scarlet, and that, as far as could be detected, no difference existed between the two mutants. There is no possibility from the pedigrees that these two mutants could have had a common origin. Scarlet, like maroon, vermilion, and many others, is a recurrent mutation.

RELOCATION OF SCARLET.

In 1921, in making some alternated stocks with which to test the effect of age upon crossing-over in the third-chromosome, Bridges found that the locus of scarlet lies about 3 units to the right rather than to the left of Dichæte.

TABLE 121.—P_1, *Dichæte* × *scarlet*; *B. C.*, F_1 *D* ♀ × *st* ♂.

Aug. 7, 1920.	D	st	D st	+
11,881	276	251	7	7
11,882	223	200	9	7
11,883	226	221	9	8
11,884	229	230	8	6
Total	954	902	33	28

In table 121 are given the results of one set of cultures raised by Bridges in preparing the alternated stocks. The percentage of recombination for Dichæte and scarlet is 3.2. In table 122 are given the results of scarlet × pink back-

TABLE 122.—P_1, *ru scarlet* × *pink* e^4; *B. C.*, F_1 + ♀ × *st p* ♂.

Sept. 7, 1920.	st	p	st p	+
11,972	284	282	14	17
11,973	254	239	7	12
11,975	158	158	8	6
11,976	150	168	2	2
Total	846	847	31	37

cross counts, for which the percentage of recombination for scarlet and pink is 3.9. In table 123 are given counts for a four-point back-cross, viz, roughoid scarlet × pink ebony^4, for which the scarlet pink recombination was 4.6 per cent. In the first experiment on crossing-over and age (unreported), there was only 1.4 per cent of recombination for Dichæte and scarlet, and in the second 1.3, both values being unexpectedly low. The standard position of scarlet on the basis of all the data is 3.4 units to the right of Dichæte and 4.2 units to the left of pink, or at 43.8, when referred to roughoid as the zero-point.

EVALUATION OF SCARLET.

This position for scarlet is about midway between Dichæte and pink; and since this section is only about 7.6 units long, this locus would be considered a relatively useless position. But it has been found that the region in the neighborhood of pink behaves very differently from the remainder of the

TABLE 123.—P_1, *roughoid h scarlet* × *peach ss ebony*^{4}; *B. C.*, F_1 + ♀ × *ru st p* e^4 ♂.

Nov. 11, 1920.	ru st p e^4		ru p e^4	st	ru st p e^4	+	ru st e^4	p	ru st p e^4		ru p	st e^4	ru st p e^4		ru st e^4	p
12,106.....	114	109	62	67	4	5	48	34	3	2	20	16	.	.	.	.
12,107.....	82	77	63	67	4	7	46	32	1	6	31	29	1	6	.	2
12,108.....	139	117	91	76	9	11	36	45	3	3	27	31	2	3	.	1
12,135.....	43	44	31	30	3	2	13	16	. .	1	10	15	.	.	1	.
12,136.....	65	51	39	36	5	3	15	22	2	1	17	15	1	1	.	1
12,137.....	49	46	30	44	2	4	15	15	3	. .	9	8	.	1	.	.
Total.....	492	444	316	320	27	32	173	164	12	13	114	114	4	11	1	4

chromosome in many respects. For example, there is an appreciable amount of double crossing-over within the Dichæte pink section. Because of these differences it becomes highly convenient to have an excellent mutant within the Dichæte pink section. Scarlet is easily and rapidly separable from the wild-type, and in viability and productivity is entirely normal.

STAR-INTENSIFIER (i-s).

(Figure 24.)

ORIGIN AND DESCRIPTION OF STAR-INTENSIFIER.

In the F_2 offspring of a mating of a supposed new mutant "ivory" to Star Dichæte, Bridges observed that many of the Star flies were of a more extreme

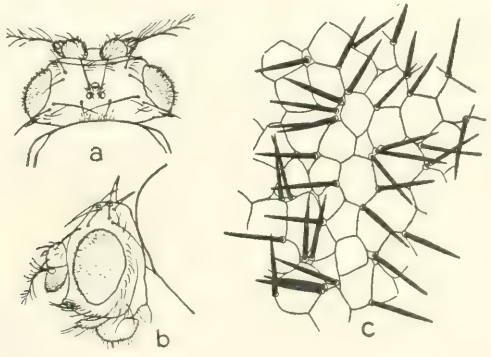


FIG. 24.—Intensified Star. (a and b) top and side views; (c) enlarged view of facets and of eye-bristles.

Star grade than usual (5,869, November 18, 1916). These intensified Stars were mainly not-Dichætes. They were bred together in pairs and in mass-culture, and an apparently pure stock was obtained, that is, stock in which all the Star flies are more extreme than standard, though the curves of normal Star and intensified-Star overlap by as much as 20 per cent of the area of each. Examination of the intensified-Star with high magnification showed that the rounding and crowding of the ommatidia are similar to that of Star but more extreme, while the hairs normally set in the angles between the facets are still more irregularly distributed. The eye as a whole is somewhat smaller than normal Star, and is relatively more narrowed from front to back, especially in the ventral part. There are slight differences in other parts of the body: the abdomen is less tapered posteriorally, and in the males may even be somewhat bulbous. The body-color tends to be somewhat more pronounced, though this effect may be only through contrast with the lighter background.

The not-Star flies of the stock cultures are completely indistinguishable from wild flies.

INTENSIFIED-STAR × DIVERGENT.

It was thought that the intensified-Star was an allelomorph of ordinary Star, and that it would be more useful than regular Star because of the greater ease of separations. Intensified-Star was used in a cross to a third-chromosome recessive, divergent-wings (see p. 182), and in F_1 (7773, October 12, 1917) there were produced 99 wild-type flies and 122 Stars which were little if any more extreme than normal Stars. The Star divergents in F_2 (table 124), from

TABLE 124.—*P_1, intensified-Star × divergent ♀; F_2, F_1 S ♂ × F_1 S ♀.*

Oct. 26, 1917.	S	int.-S	+	S dv	dv
7,930.....	56	22	48	19	11
7,931.....	90	25	63	20	20
7,932.....	114	30	62	26	14
Total..	260	77	173	65	45

matings of F_1 Stars, were all of an extreme Star type—even more extreme than the intensified Stars. This is probably an independent intensification of Star by divergent itself, for the eyes of simple divergent flies were found upon examination to be smaller than normal, rather dark, and even slightly rough. Of the Stars that were not-divergent, about a quarter were of the intensified-Star type, which is thus known to be a double mutant type—Star and a recessive specific-modifier of Star.

INTENSIFIED-STAR × DICHÆTE.

The F_2 results from the cross of intensified-Star by divergent were indecisive as to the chromosome of the specific intensifier; slightly less than a quarter of the F_2 Stars were intensified, but this result would be obtained if the locus of the intensifier were in the third-chromosome or if it were in the right half

of the second-chromosome. The fact that divergent interferes with the Star character made divergent useless in this connection. Accordingly the third-chromosome dominant Dichæte was used in a cross to intensified-Star, and F_1 Star Dichæte males were crossed to the wild-type females of the intensified-Star stock. Since these wild-type females were homozygous for the intensifier, the Star flies from this back-cross should all be intensified if the modifier is in the second-chromosome, while if the intensifier is in the third-chromosome

TABLE 125.—*P_1, intensified-Star × Dichæte; B. C., F_1 S D ♂ × + ♀ of int.-S stock.*

Nov. 28, 1917.	S D	int.-S	+	D
8,155.....	59	39	66	53
8,156.....	47	32	62	35
Total....	106	71	128	88

none of the Dichæte flies should be intensified-Star. The result of the back-cross (table 125) showed that the locus of the intensifier is in the third-chromosome.

INTENSIFIED-STAR × DICHÆTE HAIRLESS.

To find the position of the Star-intensifier in the third-chromosome an intensified-Star was crossed to Dichæte Hairless, and F_1 Star Dichæte Hairless females were crossed to intensified-Star males (table 126). The object in

TABLE 126.—*P_1, intensified-Star ♀ × D H ♂; B. C., F_1 S D H ♀ × int.-S ♂ (Star only tabled).*

Nov. 1, 1920.	i-s	D H	i-s D H	+	i-s H	D	i-s D	H
12,074.....	12	18	7	6	12	9	2	1
12,075.....	40	48	20	13	13	16	2	4
12,076.....	20	13	4	2	6	10	1	1
Total....	72	79	31	21	31	35	5	6

using intensified-Star rather than simply homozygous intensifier in back-crossing was to increase the proportion of Star flies from 50 to 67 per cent. As it is only in the Star flies that the modification is classifiable. the not-Star flies of the cross were discarded. The small classes, i. e., double recombinations, were intensified-Star Dichæte and Hairless, from which the locus of the intensifier is known to be to the left of that of Dichæte. The intensifier Dichæte recombination per cent was 22.3, which means about 24.0 as the cross-over value or distance between the loci of the intensifier and Dichæte.

INTENSIFIED-STAR HAIRY SOOTY × DICHÆTE.

This position for Star-intensifier was in the long gap between the recently found roughoid and sepia. Since this unoccupied left-end region was so long it seemed desirable to locate more exactly even this cumbersome and not very readily classifiable intensifier of Star. Star-intensifier and roughoid would interfere with each other in classification, so the nearest useful base was sepia, or hairy, which had just been located at half a unit to the right of sepia. Intensified-Star was crossed to sepia hairy sooty and F_1 Star females back-crossed to sepia hairy sooty males (table 127). This procedure furnished,

TABLE 127.—*P_1, intensified-Star × sepia hairy sooty; B. C., F_1 S ♀ × se h e^s ♂.*

Dec. 9, 1920.	se h e^s	+	se	h e^s	se h	e^s
12,206.....	87	89	3	1	52	47
12,207.....	75	88	..	..	57	38
12,208.....	131	132	..	1	79	78
12,283.....	31	50	..	1	23	24
Total.....	324	359	3	3	211	187

through crossing-over, a few hairy sooty flies which carried a third-chromosome in which the left end carrying sepia was replaced by a left end carrying the intensifier. A Star-intensifier hairy sooty stock was thus obtained; and a male from it was out-crossed to a sepia Dichæte female. The F_1 Star Dichæte females were tested by intensified-Star hairy sooty males, with the results shown in table 128, in which the distribution of sooty is disregarded and

TABLE 128.—*P_1, intensified-Star hairy × sepia Dichæte; B. C., F_1 S ♀ × int.-S h ♂.*

Jan. 3, 1921.	i-s h	D	i-s D	h	i-s h D	+	i-S	hD
12,289.....	31	37	12	11	8	7	2	6
12,300.....	49	59	17	16	4	6	4	1
12,301.....	50	47	21	14	3	4	7	6
12,315.....	20	20	10	1	6	3	2	1
12,316.....	38	31	13	6	3	2	6	6
Total.....	188	194	73	48	24	22	21	20

from which the not-Star flies are omitted. The results of table 128 are aberrant in regard to the number of double recombinations, which is nearly equal to the number of simple recombinations for the second section. This excess of doubles occurs in two cultures, Nos. 12301 and 12316, and is unaccounted for. The intensified-Star stock is known to have carried a crossing-over modifier affecting the second-chromosome, and it is possible that this modifier is also affecting the third-chromosome. The separation of intensified-

Star from Star is not very satisfactory, and it is very possible that an error has arisen from this fact. The per cent of recombination for intensifier and Dichæte was 28.3, comparable with the 22.3 of the previous experiment. But the intensifier hairy recombination per cent was nearly as great, namely, 27.5, and this would indicate a position for intensifier near, or even to the left of, roughoid. The exact position of the intensifier, therefore, remains unsettled; its mean position, on the basis of the two experiments, is approximately 10.

BENIGN.

(Figure 25.)

Dr. Mary B. Stark reported (Proc. Nat. Acad. Sci. 5, 573–580) the occurrence of a tumor that does not cause the death of the flies. The tumors were found (November 1916) in the stock of lethal-7, which causes the development of a tumor in the flies that die. The new tumor was distinguished from the lethal-7 tumor because it appeared in females as well as in males, and in adults as well as in larvæ. The tumors appear as black bodies lying free in any segment of the larva, but most frequently in the rearmost ("12 and 13"). These larvæ develop normally into flies in which the tumors are still present as large black bodies. After several generations of inbreeding of tumor-bearing flies, a stock was obtained in which the tumors could be seen in all of the individuals. This stock was freed of the lethal-7 tumor by selecting (white-eyed) flies that from the linkage relations were known to be free from the sex-linked lethal.

BENIGN × STAR DICHÆTE.

Tumor females were outcrossed to Star Dichæte males, and F_1 S D males were mated to F_1 wild-type females. Only a few of the resulting offspring had tumors, but none of these flies were Dichæte, so that a gene for tumor was shown to be in the third-chromosome. The small proportion of tumor flies suggested that the appearance of the tumor was dependent upon other genes besides those in the third-chromosome.

BENIGN × DICHÆTE HAIRLESS.

To locate the gene for benign within the third-chromosome, tumor flies were crossed to Dichæte Hairless, and F_1 Dichæte Hairless females were back-crossed to tumor males (table 129). Only 40 of the 850 back-cross flies had tumors;

TABLE 129.—*P_1, benign × Dichæte Hairless; B. C., F_1 D H ♀ × be ♂ (M. B. Stark).*

July 10, 1918.	D H	+	D	H	be	be H
Total (6 cult.).	348	276	108	78	30	10

of these, 10 were tumor Hairless, but none were tumor Dichæte. The locus of the tumor gene is thus very close to that of Dichæte, but just how close is uncertain from the smallness of the numbers on which the calculation is based.

Later unpublished work of Miss Stark shows that the locus of be–III is about 15 units to the left of Dichæte, or at 25±. A complementary gene is located

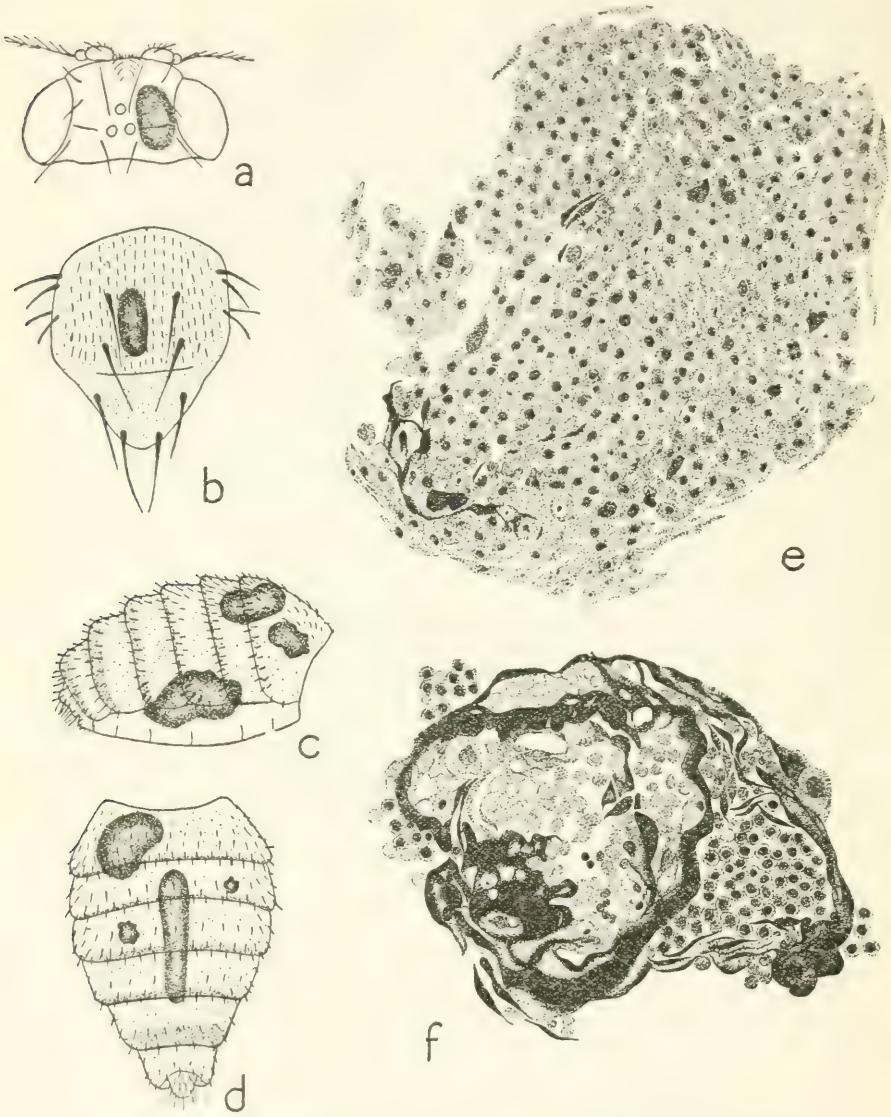


FIG. 25.—Benign tumor. a, b, c and d, non-lethal tumor present in head, in thorax, or in abdomen; e and f, sections of benign tumors.

about 10 units to the left of black; and there is probably a IV–chromosome modifier also. The inheritance is exceptionally hard to follow, and these locations are only tentative.

PINK^4 (p^4).

ORIGIN OF PINK^4.

The mutation "rose" was found by Mildred Hoge Richards (January 7, 1917) in the F_2 from the cross of scarlet to eyeless, and was then shown to be present in the eyeless stock (see Biol. Bull. 1918, 35; 199–206). "Rose" was shown to be independent of eyeless (IV), of salmon (I), and of vestigial (II), and to give a 2 : 1 : 1 : 0 linkage ratio with sooty. A "rose" by sooty back-cross gave 343 recombinations in 1,624 flies, or 21.1 per cent. Crosses of "rose" to pink gave an intermediate F_1, which in F_2 gave no wild-type. That is, "rose" is an allelomorph of pink, which is lighter than pink, being practically identical with the allelomorph peach in young flies, but not darkening so much with age. The mutant was renamed pink^4 as the fourth distinguishable mutant of the pink locus. Pink^4 conforms to the general rule of non-modification of the effects of other pinks. For example, pink^4 salmon (salmon is a light-pink allelomorph of garnet, locus 44.4 in I) is indistinguishable from salmon, which is itself practically identical in color with pink^4.

EBONY^3 (e^3).

In testing the effect of Ciii (from eosin stock, see p. 90) on crossing-over in the third-chromosome, Sturtevant mated a female carrying Ciii and a lethal in one III–chromosome and ss e^s ro in the other to a se ss e^s ro male. This culture produced 55 wild-type flies, 56 ss e^s ro, and 6 flies that resembled sooty but were somewhat darker (culture 2685, A. H. S., February 8, 1917). These dark flies were tested and were found to carry se ss e^s ro in one chromosome, and in the other Ciii, the lethal, and a new allelomorph of ebony, viz., ebony^3, that was darker than sooty but lighter than ebony. Evidently the wild-type allelomorph of ebony that was present in the Ciii lethal chromosome mutated to e^3. This mutation must have occurred in the oögonial stage of the mother, for 6 flies carrying the gene were produced. It is also probable that the mutation occurred in only one of the two third-chromosomes of the mother, namely, the Ciii lethal one, since none of the 56 ss e^s ro flies produced by culture 2685 appeared to be e^s-e^3.

Homozygous ebony^3 was not obtainable because of the presence in the same chromosome of Ciii and a lethal, but comparisons of the compounds of ebony^3 showed that the gene produced a somatic effect intermediate between sooty and ebony. The color series from darkest to lightest was found to be ee, ee^3, ee^s, e^3e^s, e^se^s, $+e$, $+e^3$, $+e^s$, $++$. Nowhere in this color series do adjacent classes fail to overlap each other, though the distinction between e^se^s and $+e$ is the greatest. Experiments using these allelomorphs should, of course, be so planned that only separable classes would occur.

Ebony^3 was discarded, for the presence of the lethal made the labor of maintaining the stock too great, and the locus was satisfactorily represented by sooty and by ebony^4.

DIVERGENT (dv).

(Figure 26.)

ORIGIN OF DIVERGENT.

In determining the locus of a second-chromosome recessive, telegraph, by means of a back-cross with Star (intensified-Star), Bridges found that a wing

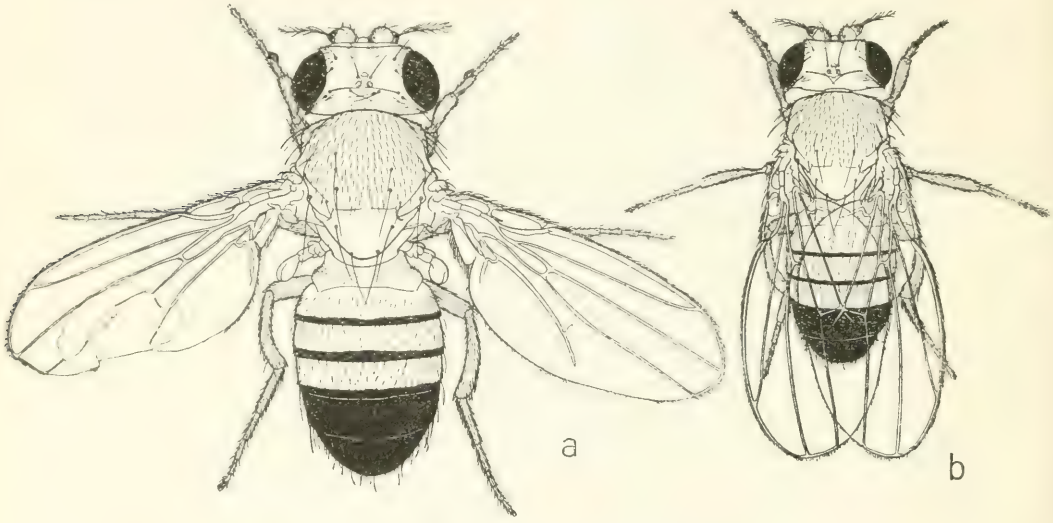


FIG. 26.—(a) Divergent wings; (b) dwarf-b.

mutant resembling spread was present (7251, June 13, 1917). A stock of "divergent" was obtained, which bred with difficulty, since the females were often sterile and usually showed low productivity. There was a heavy mortality, especially in mass-cultures.

DIVERGENT × HAIRLESS.

A divergent male was crossed to a Hairless female, and three F_2 pairs were mated (table 130). The results showed that divergent is in the third-chromo-

TABLE 130.—*P_1, divergent ♂ × Hairless ♀; F_2, F_1 H ♂ × F_1 H ♀.*

Aug. 6, 1917.	H	+	dv	dv H
7,473.....	90	21	23	14
7,474.....	194	27	59	41
7,475.....	187	25	31	27
Total....	471	73	113	82

some, and accordingly a few divergent Hairless back-cross cultures were raised to determine its locus (table 131). The recombination per cent for table 130

is 39.4, and for table 131 is 35.6, but the classes were so variable in their relation to one another that very little reliance is to be placed on these data. It is probable from a comparison of the mean divergent Hairless recombination

TABLE 131.—*B. C., H ♀ from table 130 × divergent ♂.*

	dv	H	dv H	+
7,559.....	64	89	50	41
7,564.....	63	68	27	45
7,587.....	28	47	17	20
7,588.....	33	41	13	26
7,589.....	3	43	5	11
7,590.....	5	77	10	27
7,645.....	28	129	23	27
Total....	224	494	145	197

per cent of 36.6 with other standard per cents that the locus of divergent is some 46 units from Hairless, and it seems slightly more probable that it is to the left, i. e., at 24±. More recent data show that the locus is at about 20.0.

A cross of divergent by intensified-Star (table 124) showed that divergent acts as an even more extreme modifier of Star than does the specific modifier.

CIIIP.

In attempting to find the locus within the third-chromosome of a gene that tended to increase the number of bristles on the scutellum, Fernandus Payne crossed a female having extra scutellar bristles to a seple (sepia spineless kidney sooty rough) male, and raised F_2 from pairs of F_1 flies with extra scutellar bristles (reported in Indiana University Studies No. 36, March 1918; date of F_2, July 1917). Disregarding the presence of extra bristles, the F_2 flies were largely of two classes, viz, wild-type (2701) and seple (609), with only a few flies representing recombination for the elements of seple. That is, a dominant cross-over-reducer was present in the F_1 female. The recombinations were 7 se, 8 ss k e^s ro, and 2 ss. The percentages of recombination were: se ss = 2.7, ss e^s = 0.3, e^s ro = 0.0, as compared with the standard per cents of 30, 12, and 19, respectively. The only previously known dominant cross-over variation in the third-chromosome was CIII. CIII gave similar values for ss e^s and e^s ro, but gave 20± for se ss instead of 2.7. This is a clear difference in the region affected, and also the origins of the two were independent.

Since the scute stock, derived from the sixth generation of the extra-bristle stock, did not carry CIIIP, it is probable that the mutations to CIIIP occurred during the later course of the selection. Lance, a second-chromosome wing mutant, appeared December 5, 1916, in the sixteenth generation of selection. This mutant carries the CIIIP factor. Since scute (or reduced) appeared April 15, 1916, in the sixth generation of the same line, CIIIP must have occurred some time between the sixth and sixteenth generation of selection.

EBONY^4 (e^4).

(Plate 2, Figure 4.)

ORIGIN OF EBONY^4.

In September 1917, A. F. Huettner brought from Mitchell, South Dakota, a fresh wild stock of *Drosophila melanogaster*. In this stock Sturtevant found several very dark flies (September 27, 1917) that appeared much like ebony, and from which a pure-breeding stock was derived. It is clear that the mutation had occurred in a wild fly in nature, for the mutant was represented by several flies in the fresh stock.

EBONY^4 × STAR DICHÆTE.

A "dark" male was out-crossed to Star Dichæte, and an F_1 Dichæte male was back-crossed to a "dark" female. The result (4119, A. H. S.) was a total of 52 Dichæte and "dark" flies with no recombinations, from which it is known that the gene for "dark" is in the third-chromosome.

A female back-cross gave 66 recombinations in a total of 169 (4116, A. H. S), showing that the locus of "dark" is about 39 units from Dichæte.

EBONY^4, ALLELOMORPH OF EBONY.

Dichæte and sooty usually gave this same per cent of recombinations, and since "dark" resembled ebony closely, a cross of "dark" by cream–III ebony was made. The cross produced ebony flies in F_1, from which it is known that "dark" is either ebony, by a new mutation, or else an allelomorph of ebony. Examination of "dark" showed that it is probably not identical in character with ebony, but has a somewhat lighter colored abdomen. Ebony^4 also gives a slightly darker heterozygote than does ebony. The mutant ebony^4 is very easily classifiable, and has been used rather largely instead of sooty in the later work. The viability of ebony^4 is rather poor.

All the ebony stocks carried CIII, but ebony^4 was free from CIII, as the large amount of recombination of Dichæte and ebony^4 showed.

PALE-TRANSLOCATION.

(Plate 1, Figure 14.)

In preparing some stocks of sex-linked characters for working with a lethal tumor (lethal–7), Bridges found that in one culture (7940, October 16, 1917) there were present the expected eosin flies, and in addition flies with a light yellow or cream eye-color (plate 1, fig. 14). In out-crosses of "Pale" males to eosin females about a third (35.5 per cent) of the offspring, both sons and daughters, were Pale. That is, Pale was found to be not-sex-linked, and to behave as a dominant, except that the frequency was lower than expected for a dominant. When Pale was bred to Pale, about two-thirds (66 per cent) of the offspring were Pale, which suggested that homozygous Pale is lethal, as is the case with Star, Dichæte, and most of the other dominants of *Drosophila*.

PALE A SPECIFIC DILUTOR OF EOSIN.

A Pale female was out-crossed to a wild male, and it was found that none of the F_1 females were different from wild-type, although a third of the sons

were Pale and two-thirds eosin; that is, Pale is a specific modifier of eosin, similar to Cream–II (Bridges, 1919). The F_1 females were crossed to Pale brothers, and again none of the not-eosin females or males were diluted. Among the eosin flies a third were Pale in some cultures and two-thirds in the others. These latter cultures came from females that were "not-eosin Pales."

PALE × STAR DICHÆTE, MALE BACK-CROSS.

A Pale male was out-crossed to an eosin Star Dichæte female, and F_1 Pale Star Dichæte males were out-crossed to eosin females. There were produced only three classes, viz, 596 P, 629 S D, and 512 S. None of the Pale flies were Star or Dichæte, which proved that Pale depends upon the simultaneous presence of two producers, one located in the second, the other in the third chromosome. We may call these interdependent producers Pale–II and Pale–III (symbols, PII and PIII).

PII A DOMINANT LETHAL, EXCEPT IN PRESENCE OF PIII.

In the above back-cross, a third of the flies were Pale, but two-thirds were Star and only one-third were Dichæte. Both Star and Dichæte are of normal viability. These ratios called for special explanation. The F_1 male should produce four classes of gametes in 1:1:1:1 ratio, viz, PII PIII, S D, S PIII, S PII D. No class corresponding to the PII D gamete was realized. It was not that this class was phenotypically the same as some other class, for none of the other three classes were increased above their proper 1:1:1 ratio. It was rather that the Dichæte class perished, through the action of PII. But when PII was present together with PIII, there was no lethal effect. PIII, without PII, was present in the Star class, showing that PIII produces no dilution, and is not lethal, although of somewhat reduced viability.

LOCUS OF PII.

Nine female back-cross cultures of (eosin) Star × Pale gave totals of 470 P, 383 S P, 720w^e S, 763w^e, of which the S P and w^e classes are recombinations and the P and w^e S original combinations, giving 49.1 per cent of recombination. Some of the recombination S P females were out-crossed again to eosin males, giving totals of 214 S P, 189 P, 403 w^e, 3330 w^e S, corresponding to 45.5 per cent of recombination. That is, the locus of PII is far to the right of Star.

A back-cross of Pale × eosin arc morula gave four recombinations for Pale and arc in a total of 4,254 flies, corresponding to 0.1 per cent of recombination; and the relation of Pale to morula showed that the position of Pale–II is to the right of that of arc.

LOCUS OF PIII.

Eosin Dichæte Hairless females were crossed to Pale males and F_1 D H P females out-crossed to eosin males, giving 676 P, 151 D P, 11 D H P, 2 H P; 620 D H, 605+, 132 D, 150 H. Among the Pale flies the smallest class, or

double-recombination class, was H P, which shows that the order of loci is D H P. There was 1.5 per cent of recombination for H and Piii.

Since Hairless and ebony normally give about 1.2 per cent of recombination, the next locus used in determining the position of Pale–iii was ebony in a five-point back-cross (D P × ss e^s ro), giving 892 D P, 157 P, 55 ss P, 55 ss e^s P, 1 D P ro, 2 D ss P, 1 D ss e^s P, 2 P ro; 615 D, 810 ss e^s ro, 136 D ss e^s ro, 132+, 36 D e^s ro, 30 ss, 3 D ro, 8 ss e^s, 3 D ss, 2 e^s ss, 2 ro. The data show that the locus of Piii is between those of ebony and of rough; but that the amount of recombination in that region has been reduced from about 20.4 (standard) to 0.8.

PIII A "DUPLICATION" OF THE PLEXUS-SPECK SECTION OF CHROMOSOME-II.

If Pale is out-crossed to eosin speck, half of the not-Pale offspring should carry Piii without Pii. Matings of the F_1 not-Pale females by speck males give two types of cultures, the normal type, in which the ratio of speck to not-speck is the standard 1 : 1 of a back-cross, and cultures in which the ratio is 1 speck : 3 not-speck. In these latter cultures half of the speck flies are prevented from showing the speck character by the presence of Piii. Experiments similar in principle to the above have shown that Piii has this "covering" effect upon plexus, brown, speck, morula, balloon, purpleoid, blistered, and lethal–iia, that is, upon all mutants whose loci are to the right of that of arc. In the case of lethal–iia, half the flies that would have died are saved, and are recognizable through the use of recessives linked to the lethal. Plexus differs from the others in that it is not entirely converted into wild-type, but shows as a weak plexus that is clearly distinguishable from standard plexus. This situation is met by the assumption that Piii is in reality a fragment of the second-chromosome, covering the section from arc to the end of the chromosome, that has been transferred bodily to the third-chromosome. The translocation occurred in a fly all of whose genes in this section were wild-type, so that a fly homozygous for speck and carrying Piii is really triploid for the speck locus; and the two recessive speck genes are recessive to their wild-type allelomorph carried by the third-chromosome. In the case of plexus, the two plexus genes are partially dominant over the one wild-type allelomorph. Analogous cases of "duplication" of sections of the X–chromosome had been studied previously by Bridges, and these gave the clue to the present peculiarities of inheritance.

PII A SUPPRESSOR OF CROSSING-OVER IN SECTION TO RIGHT OF ARC.

In females heterozygous for Pii and for recessives within the affected region, back-cross tests show that there is no crossing-over throughout this section. This fact is in conformity with the analysis of the nature of Pii, viz., that Pii is the deficiency of the above section. In a fly heterozygous for Pii there would be one normal II–chromosome, carrying the recessives being tested, and another chromosome shorter than normal by this whole section. Synapsis and crossing-over would be impossible, unless Piii could be involved.

LETHAL EFFECTS OF PII AND PIII.

Tests have shown that flies differing from normal by being heterozygous for PIII are only about 80 per cent as viable as normal flies. Flies homozygous for PIII are entirely unable to live. Flies heterozygous for PII are unable to live unless PIII is also present in heterozygous form, in which case their viability is normal, or unless PIII is homozygous, in which case the viability is again about 80 per cent of normal. Flies homozygous for both PII and PIII are lethal. This last fact, together with the Pale color of the heterozygous PII, PIII eosin flies, may be interpreted to mean that PII–deficiency is slightly greater than PIII–duplication, in which case PII, like deficiencies in general, would be lethal when homozygous, and would show character changes when heterozygous. The way in which these character changes may be brought about through changes in the balance of genes is treated by Bridges elsewhere (Am. Nat., Jan.-Feb., 1922).

THE ORIGIN OF THE TRANSLOCATION.

The locus of PIII was found to be in the right limb of chromosome–III, between ebony and rough. That this locus was not at the end (that is, not to the right of rough and others) or at the middle (near pink) gave a clue as to the possible mode of origin of this translocation. If the two third-chromosomes synapse with their free ends lying together (bouquet stage) it might happen that the right-hand end of one of the second-chromosomes should sometimes lie between the right-hand ends of the two third-chromosomes. Continuation of the synapsis of the pair of second and of the pair of third-chromosomes would result in two pachytene strands which are interlocked by half their thickness at a single point near the right end of each. We may suppose that in the forcible separation of these interlocked chromosomes the second-chromosome was torn in two and the end carried away by the third-chromosome.

SEMI-FORKED.

In February 1918, Lancefield (Am. Nat., 52, 462-464) found in a culture in which all the females should be heterozygous for the sex-linked recessive forked-bristles, that about a quarter of the females showed a character intermediate between standard forked and the wild-type. The "semi-forked" character was found to be due to an independently inherited autosomal recessive, which intensified the action of the forked gene so that flies heterozygous for forked and homozygous for the intensifier became modified to "semi-forked." Flies heterozygous for forked and simply heterozygous for the intensifier were not modified. Flies homozygous for the intensifier but not carrying forked were wild-type. That is, the mutant is a recessive specific modifier of forked, similar in action to the specific intensifiers of eosin (the creams, see p. 118).

A semi-forked female was out-crossed to a Star Dichæte male, and the F_1 S D males were back-crossed to semi-forked females. Among the progeny of the cross none of the Dichæte flies were semi-forked, while all of the not-Dichæte flies were such. The gene of the intensifier is thus in the third-chromosome, but its locus within that chromosome has not been determined.

GLASS (gl).

(Plate 1, Figure 15; Plate 3, Figure 2; Figure 27.)

ORIGIN OF GLASS.

In the stock of sable-duplication garnet, Muller found some flies with colorless eyes having a smooth surface, which he called "glass" (February 1918). A pure-breeding stock was secured without difficulty; and this stock was freed

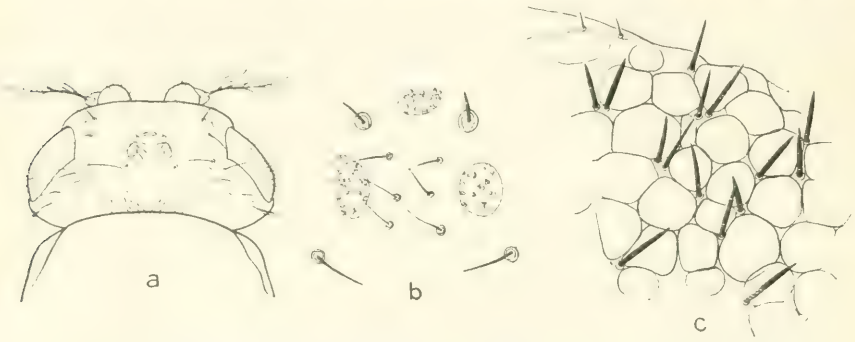


FIG. 27.—Glass eye. (a) top view; (b) enlarged view of ocelli; (c) enlarged view of central region of eye.

from sable-duplication and garnet by out-crossing and extracting. It then became apparent that the glass eye itself carried some color, the colorless condition of the eyes first observed being due to the interaction of garnet and glass.

DESCRIPTION OF GLASS.

The character glass is an eye variation in which the facets of the eye have lost their separate character, and form a continuous smooth, glassy surface. The area of the eye is only about half that of the wild-type; the reduction being mainly in the antero-posterior direction and most pronounced at the bottom and top of the eye, so that the eye is a narrow lozenge, or diamond-shaped. The color of the eye is also greatly reduced and altered. In the superficial layer the pigment is practically absent, or reduced to a diffuse straw-colored tinge. The deeper parts are pigmented more heavily, so that in surface view the eye has a colored center surrounded by a broad colorless rim. The eye is markedly sex-limited in its manifestation, the color in the female being reduced much more than in the male. In the female the centrally located deeper-lying pigment is often reduced to little more than a tinge, like that in the periphery. In the male the pigment is not reduced so much, the color being usually a vermilion or orange. The color in the periphery seems largely in solution, while the color in the center is granular, but irregularly distributed as though eroded and etched into, and in the female nearly dissolved away.

THE OCELLI OF GLASS.

In making the drawing for the character glass, Miss Wallace noticed that the ocelli are also modified (fig. 27b), their surface being covered with very tiny mushroom-like elevations.

REDISCOVERY OF GLASS.

In the spring of 1919, O. L. Mohr also discovered glass in the sable-duplication garnet stock; and he worked out the chromosome and locus without knowledge of the work of Muller. Mohr kindly provides us with the following summaries of his linkage data (table 132):

TABLE 132.—*Female B. C. data involving glass, provided by Dr. O. L. Mohr.*

D × gl	D		gl	D gl		+	Total.	Recomb. per cent.		
July 13, 1919.	169		149	67		73	458	30.5		
D H × gl	D H	gl		D gl	H	D	gl H	D gl H +	D gl	gl H	D H
Oct. 14, 1919.	1,502	1,383		271	334	124	110	7 8	16.6	6.7	22.5
ss gl × Δ	ss gl	Δ		ss Δ	gl	ss gl Δ	+	Total	ss gl	gl Δ	ss Δ
Jan. 20, 1920.	1,072	1,166		69	70	40	49	2,466	5.6	3.6	9.2
ss gl Δ × +	ss gl Δ	+		ss	gl Δ	ss gl	Δ	Total	ss gl	gl Δ	ss Δ
Feb. 18, 1920.	324	393		23	23	16	15	794	5.8	3.9	9.7
ss Δ × gl	ss Δ	gl		ss gl	Δ	ss	gl Δ	Total	ss gl	gl Δ	ss Δ
Feb. 18, 1920.	593	542		17	18	22	19	1,211	2.9	3.4	6.3

The first of Mohr's experiments, a glass by (Star) Dichæte female back-cross, showed that glass is in the third-chromosome, and considerably removed from Dichæte.

The second experiment, glass by Dichæte Hairless, showed that the locus of glass is between those of Dichæte and Hairless, and some 7 units to the left of Hairless.

The best mutants in the region in which the Dichæte Hairless test placed glass were the primary base spineless, about 12 units to the left of Hairless, and the secondary dominant Delta, some 3 units to the left of Hairless. Glass should be about midway between these loci. A spineless glass double recessive was made, and of the four possible back-cross tests three were carried out, namely, as gl × Δ, ss gl Δ × +, and ss Δ × gl. The first two agreed closely with one another and with the results of the gl × DH back-cross, while the third gave a somewhat lower value for the spineless glass section.

THE LOCUS OF GLASS.

The data of Muller, those of Mohr, and those obtained by Bridges in the ss bx gl experiments (see p. 144) are combined in calculating the locus of glass. The calculation of the primary bases (p. 24) places Hairless at 69.5 and spineless at 58.5, while Delta falls at 66.2. The locus of glass with relation to these bases is at 63.1.

EVALUATION OF GLASS.

Glass is very easy to classify, but interferes with the use of other eye-colors, such as pink and claret, and probably also with "moruloids," such as rough

and roughoid. It is normal in viability and productivity. Its locus is in the section between spineless and Hairless, in which there is only negligible double crossing-over. The principal use of glass is in experiments made to furnish more accurate data for the location of bithorax, and other mutants in that region, and in testing special conditions, such as deficiencies, etc.

ROTATED-ABDOMEN.*

(Figure 28.)

The mutant rotated-abdomen appeared (July 28, 1918) among the offspring of a pair of flies which were a part of an experiment dealing with the translocation of a piece of the second-chromosome from its normal position at the end of the second-chromosome to a new position as a part of the third-chromosome. In this experiment the translocation gave rise to a dominant char-

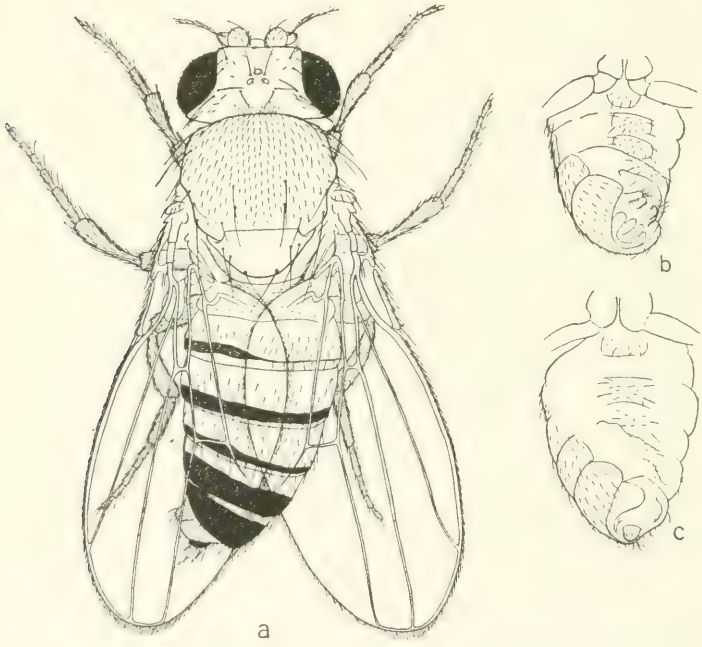


FIG. 28.—Rotated abdomen. (*a*) dorsal view of female; (*b*) ventral view of male; (*c*) ventral view of female.

acter "Pale" located at the right end of the second-chromosome. A Star Pale Dichæte male (from a cross of a female carrying Pale to a Dichæte Star male) was crossed to a yellow (sex-linked recessive) female showing none of these dominants, but taken from a stock related to Pale. Rather less than a quarter of the offspring of this pair had abdomens that were twisted at the

* An account of rotated-abdomen was furnished to Sumner and Huestis who published it in connection with their study of bilateral asymmetry. (Genetics 6: 445-485.)

end. *The rotation was in all cases to the left,* and was through from 60° to 90°, with no apparent overlap with the normal unrotated condition. The rotation was equally frequent among males and females. Neither of the parents of the culture had shown this character, which was evidently a simple recessive.

The rotated character was distributed at random with respect to sex, yellow, Star, and Pale, but not one of the Dichæte flies was rotate; that is, the gene for rotate is not sex-linked, not in the second-chromosome, but is in the third-chromosome. This conclusion was certain because the cross was of the male back-cross type, which gives decisive evidence from the fact of no crossing-over in the male.

The viability of the mutant was not good, as evidenced by the low percentage. This low percentage was known to be due to a viability change and not to an overlapping of the normal, since no doubtful intermediate specimens were ever found, and also since the ratio among the normals was such as to correspond to a third-chromosome lethal. Both sexes of rotated flies proved sterile, probably because of difficulty in mating. Aside from the rotation no structural change was observed. The race was continued for some time by breeding together heterozygous brothers and sisters, but was finally lost.

The unusual feature of rotated-abdomen was its asymmetrical nature, the abdomen being invariably twisted toward the left. Only one other such character has been found—a twisting of the male genitalia, also to the left, and often so extensive as to reverse the direction of the penis. The origin of asymmetrical structures as Mendelian characters is a fact, although the steps by which such asymmetry is brought about are unknown.

MAHOGANY (my).

(Plate 1, Figure 13.)

ORIGIN OF MAHOGANY.

In working out the effects of a second-chromosome lethal that is probably a deficiency (lethal–IIa), Bridges found that approximately a quarter of the flies of one culture (8951, November 13, 1918) showed a brownish-red somewhat translucent eye-color that was called mahogany. A pure stock of black curved mahogany was secured.

MAHOGANY × STAR DICHÆTE.

A male back-cross of mahogany with Star Dichæte showed that the locus of mahogany is in the third-chromosome. From the back-cross progeny a Star mahogany male was out-crossed to a wild female; and an F_2 culture was raised from F_1 Star flies. This procedure furnished a stock of mahogany (in F_3) which was free from the second-chromosome recessives black and curved and from the dominant Star.

MAHOGANY × DICHÆTE HAIRLESS.

A female back-cross of mahogany by the double dominant Dichæte Hairless (table 133) showed that the locus of mahogany is between D and H and

TABLE 133.—*P_1, mahogany × Dichæte Hairless; B. C., F_1 D H ♀ × my ♂.*

Jan. 13, 1919.	D H	my	D my	H	D	my H	D my H	+
9,273.....	63	78	11	5	21	21	8	6
9,314.....	45	44	3	4	17	20	2	1
9,328.....	85	85	5	4	33	34	3	1
Total.....	193	207	19	13	71	75	13	8

some 9 units to the right of Dichæte, or at about 49.5. This location is not very accurate, for mahogany proved rather hard to classify, overlapping the wild-type considerably, especially in the males. The apparent number of double recombinations far exceeded the normal ratio.

NON-MODIFICATION OF EOSIN.

The fact that some 10 specific or disproportionate modifiers of eosin were known, suggested that the presence of eosin might make the difference between mahogany and not-mahogany flies more marked; eosin mahogany might be clearly separable from eosin. A stock of eosin mahogany was made up, but it was found impossible to distinguish this from the simple eosin stock. A similar failure to modify eosin has since been found to hold for several other brownish eye-colors, the most striking case being that of the brownish pink second-chromosome recessive "brown." Brown is far lighter in tone than is ruby^2, yet eosin ruby^2 has only a pale yellow tinge. Several distinct classes of eye-colors are now separable by means of their type of interaction.

MAHOGANY SPINELESS SOOTY × DICHÆTE DEFORMED.

The mahogany × D H results seemed to locate mahogany farther to the right of Dichæte than any of the other numerous genes whose loci are near to pink. That region is especially interesting in several respects, and an attempt was made to locate mahogany more accurately by means of Deformed, which is within a unit of pink and to the left of spineless, which is the left-most locus of the region lying to the right of the sensitive region about pink. A mahogany spineless sooty stock was made up, and a back-cross was made with it and the double dominant Dichæte Deformed (12469). The classification of mahogany proved too difficult, but the indications were that the locus of mahogany is about 3 units to the right of Deformed. Perhaps some special method of handling this mutant will be found by which it can be used to investigate the region in question.

COMPRESSED (cp).

(Figure 29.)

ORIGIN OF COMPRESSED.

The stock of the new recessive mutant aristita had become reduced to a few flies that seemed sterile *inter se*. In an effort to save the stock these flies were out-crossed to flies that carried Dichæte and Hairless in one third-chromosome and spineless and white-ocelli in the other. One such mating produced a very few offspring; and since all the other aristita flies had died meanwhile, these were the only means of recovering the stock. The above third-chromosome mutants had been used in the cross to aristita on the basis of a previous indication that aristita was not in the second-chromosome and was therefore probably in the third. But it was now realized that this supposed indication had been untrustworthy, and accordingly a definite test of the point was proposed. Some of the F_1 flies, heterozygous for aristita and for spineless and white-ocelli, were out-crossed to Star Dichæte, and quite a number of F_1 Star Dichæte pairs were mated. One such pair gave aristita, and in such relations that it was evident that the locus of aristita is in the second-chromosome close to that of Star (culture 8966). In a sister culture Bridges found 4 flies with a curiously flattened head and very small round eyes (8967, November 27, 1918; 86 D, 6+, 3 cp). This character was called compressed.

DESCRIPTION OF COMPRESSED.

The ventral part of the head of the "compressed" flies appears crushed in, so that the head in side view is very flat from top to bottom. The cheeks are more ventral than lateral, and each bears a tuft of small bristles formed by the crowding together of the bristles that normally occupy a much larger area. The eyes are very small, and are crowded to the posterior dorsal part of the area they usually occupy. They are round and smooth, unlike the eyes of kidney or Deformed (which are similar in location and size and in the bristle tuft on the cheek). The aristæ are crumpled and shortened. The humeral areas are elevated into plateaus, with the humeral bristles distorted. The wings may be extended or drooped.

DICHÆTE × COMPRESSED.

In the culture in which the compressed character was first observed (8967) there were 86 Dichæte, 6 not-Dichæte, and 3 compressed. The distribution of the characters spineless and white-ocelli was not followed. The fact that nearly all the flies, instead of only 67 per cent. were Dichæte, showed that there was present in the opposite chromosome something which was killing off homozygous not-Dichætes. This could be a recessive lethal; or it could be the compressed, if compressed were itself a surviving semi-lethal. The compressed flies were themselves not-Dichæte, which would agree with this position for its gene. On the assumption that compressed is a semi-lethal in the not-Dichæte chromosome, the number above indicates 13.0 per cent of recombination for Dichæte and compressed. ($D = 2o + r = 86$; $+ = r = 6$; $cp = o = 4$, $D\,cp = r = 0$; equivalent to 6 recombinations in a total of 46). Of course, the reliability of this determination is poor, and the numbers are small.

Two of the compressed flies were males, and these were crossed to their Dichæte sisters. One mating failed, but the other (9037, table 134) gave a

TABLE 134.—*P_1, compressed ♂×Dichæte ♀; B. C., F_1 D ♀×cp ♂.*

Dec. 9, 1918.	D	+	cp	D cp
9,037	67	7	29	4
9,974	18	1	7	.....
9,975	14	.....	3	.....
9,998	25	2	11	.....
10,086	50	3	37	5
10,087	3	1	8	.....
10,089	39	1	23	.....
10,215	123	5	19	3
Total	339	20	137	12

confirmation of the assumption that compressed is a semi-lethal, situated some 10 units from Dichæte.

In seeking to extend the Dichæte by compressed back-cross, it was found that compressed males are very poor breeders. This difficulty was surmounted by using, instead of a homozygous compressed male, a male heterozygous for compressed and carrying in the other third-chromosome the dominant Hairless. Since there is no crossing-over in the male, all offspring from such a cross that do not show Hairless are known to have come from the compressed-bearing sperm, and constitute valid back-cross material. The remainder of the flies of table 134 are the not-Hairless progeny from such matings. There were 32 recombinations in a total of 508 flies, or 6.3 per cent, somewhat lower than the value found earlier.

COMPRESSED × HAIRLESS.

A compressed by Hairless F_2 result (table 135) showed 28.1 per cent of recombination for compressed and Hairless, which left it undecided whether

TABLE 135.—*P_1, compressed ♂×Hairless ♀; F_2. F_1 H ♀×F_1 H ♂.*

Feb. 10, 1919.	H	+	cp	cp H
9,376	23	5	2	.....
9,381	115	15	16	6
9,382	109	23	26	8
Total	247	43	44	14

compressed is to the right or left of Dichæte, with the probability in favor of the right.

COMPRESSED SPINELESS WHITE-OCELLI × HAIRLESS.

Although the distribution of spineless and white-ocelli had not been followed in the previous experiments, those characters had been seen to be present in most of the compressed flies. Use was now made of them in locating com-

pressed more accurately. Counts were made including spineless and white-ocelli in 4 cultures of F_2 from compressed (spineless white-ocelli) by Hairless (table 136). From the classes it is apparent that the locus of compressed is

TABLE 136.—*P_1, compressed spineless white-ocelli × Hairless; F_2, F_1 H ♀ × F_1 H ♂.*

Feb. 13, 1919	H	ss wo	wo	H wo	ss H	cp ss wo	cp H	cp ss H	cp ss	cp H wo
9,377..	99	7	8	6	.....	2	.....	.....	1	1
9,467..	66	6	6	4	.....	.....	.....	2	1	.....
9,496..	114	14	12	8	1	9	7	1	3	.....
9,515..	106	3	3	6	2	.....	2	.....	.....	.....
Total.	385	30	29	24	3	11	9	3	5	1

some 12 units to the left of spineless—a location that is in agreement with 6± units to the right of Dichæte. Back-cross results for these 4 loci were secured in 2 cultures by using males that were Dichæte in one third-chromosome and compressed spineless white-ocelli in the other (table 137). The Dichæte offspring of the cross were dicarded.

TABLE 137.—*P_1, compressed spineless white-ocelli × Hairless; B. C., F_1 H ♀ × cp ss wo ♂.*

March 17, 1919.	H	ss wo	wo	H wo	cp ss wo	cp H	cp ss H
9,570.....	10	.....	3	1	.....	.....	.....
9,572.....	47	5	10	1	5	5	3
Total.....	57	5	13	2	5	5	3

COMPRESSED SPINELESS WHITE-OCELLI × DICHÆTE DELTA.

The location of compressed with respect to Dichæte and spineless was tested further by using males that were cp ss wo in one third-chromosome and H

TABLE 138.— $\frac{D}{cp}\ \frac{\Delta}{ss}\ \frac{}{wo}$ ♀ × $\frac{H}{cp}\ \frac{}{ss}\ \frac{}{wo}$ ♂ (*H flies omitted*).

April 16, 1919.	D Δ	Δ	D ss wo	D wo	D Δ wo	ss wo	wo	D	ss	cp ss wo	cp Δ	cp ss
9,643...	17	1	1	3	2	.....	.....	.....	.....	1	.....	.....
9,669...	26	....	2	1	9	.....	1	.....	.....	3	....	1
9,705...	57	4	6	6	7	2	2	....	1	7	....	.....
9,706...	38	5	5	4	6	1	1	.....	.....	3	1	1
9,739...	24	2	2	6	2	.....	.....	1	.....	1	1	2
9,749...	22	1	2	4	3	.....	.....	.....	.....	3	1	.....
9,805...	23	4	2	3	1	.....	.....	.....	.....	.....	.....	.....
Total..	207	17	20	27	30	3	4	1	1	18	3	4

in the other for testing females that were cp ss wo in one and D Δ in the other (table 138). The Dichæte compressed recombination per cent was 8.1 and the compressed spineless per cent was 7.7.

COMPRESSED DILAPIDATOR.

In the above cross a curious relation that had been partly seen in previous crosses came sharply to light, namely, that there was in the cp ss wo chromosome another mutant gene which by itself produced little effect, but which made compressed flies still more inviable. The double-mutant type was pale in color, with thin, ragged wings and atrophied wing muscles, so that the thorax contained a large sinus with bubbles. The locus of this "dilapidator" was shown by the cross-over relations to be between those of spineless and white-ocelli and to the right (probably) of Delta. Hence, the cross-over flies compressed spineless not-white-ocelli were relatively robust.

COMPRESSED SPINELESS DILAPIDATOR WHITE-OCELLI × DICHÆTE.

The action of the dilapidator was still clearer in the next experiment, which was the same as the above, except for the omission of Delta (table 139). The

TABLE 139.— $\frac{D}{cp \quad ss \quad wo}$ ♀ × $\frac{H}{cp \quad ss \quad wo}$ ♂ (*H flies omitted*).

May 22, 1919.	D	+	D ss wo	D wo	ss wo	wo	D ss	ss	cp ss wo	D cp ss wo	cp	cp ss	D cp	D cp ss	cp wo	D cp wo
9,883..	45	2	3	9	.....	....	1	....	3	.....	9	.....	.....	1	.....	.....
9,884..	45	8	3	7	1	....	1	....	4	.....	9	2	.....	.....	.....	.....
9,885..	47	5	6	7	.....	.....	.....	.....	11	.....	2	4	.....	.....	.....	1
9,887..	66	6	8	17	.....	.....	2	....	5	.....	9	3	1	.....	1	.....
9,888..	38	4	8	7	1	....	.....	1	8	.....	9	3	1	.....	4	1
9,889..	47	3	2	7	2	1	.....	.....	2	1	6	2	2	.....	.....	.....
9,890..	40	4	1	3	.....	.....	1	....	3	.....	5	4	.....	.....	.....	.....
Total.	328	32	31	57	4	1	5	1	36	1	49	18	4	1	5	2

Dichæte compressed recombination per cent was 8.3, and the compressed spineless 8.9, giving nearly the same relations as before. The effect of the dilapidator is clearly seen in the numerical results, for the compressed recombination class is 49, far in excess of the original class of 36. Likewise, the compressed spineless not-white-ocelli class is high. All classes in which compressed and white-ocelli remain together are low, since they carry the dilapidator.

Since the locus of the dilapidator is to the right of spineless, the compressed not-spineless cross-over chromosome should be free from the dilapidator. Accordingly, a compressed male (see table 139) was out-crossed to the multiple "peple," and F_1 wild-type females were again crossed to peple. The sooty rough cross-over flies should be compressed sooty rough in one chromosome and peple in the other. A stock of cp e^s ro was secured, and was maintained in heterozygous form by mating such males by peple females each generation. No work with this stock has as yet been done, but it will be necessary to find how the dilapidator-free compressed behaves, since the linkage results indicate that the location is a very favorable one. The final calculation of the position of compressed

on the basis of all the data gives 8.1 units to the right of Dichæte, or 10.0 units to the left of spineless.

DELTA (Δ).

(Figure 29).

ORIGIN OF DELTA.

In order to test out a new dominant mutant "Lobe" that had arisen, it had been crossed to Dichæte, and F_1 Lobe Dichæte female had then been mated to a Star black male. Among the offspring of the latter mating, Bridges found

FIG. 29.—Delta.

a single male whose wings showed a broadening out of each longitudinal vein where it joined the marginal vein (8958, November 30, 1918).

DOMINANCE OF DELTA.

The occurrence of a new mutant character in a single fly among the progeny of a pair mating indicates that the mutant is sex-linked or a dominant. The latter proved true; for the Delta male, when out-crossed to Hairless, gave in F_1 half the offspring Delta and the other half not-Delta (9107).

CHROMOSOME OF DELTA.

The Delta male had had a "Star" eye, and all the Delta F_1 flies were also "Star," apparently showing that Delta is in the second chromosome. The mutation to Delta had apparently occurred in the Star black chromosome of the father of culture 8958. Several of the F_1 "Star" Delta (not-Hairless) females were out-crossed to black males in order to find the locus of Delta

within the second-chromosome by means of a three-point back-cross (table 110). In the back-cross progeny all of the 444 Deltas were "Star," which

TABLE 110.—*P_1, "Star" Delta black × wild; B. C., F_1 "S" Δ ♀ × b ♂.*

Dec. 28, 1918.	"S" Δ b	+	"S" Δ	b	Cont.	"S" Δ b	+	"S" Δ	b
9,226.....	59	43	44	41	9,240....	32	46	43	40
9,227.....	12	5	22	10	9,250....	19	40	27	30
9,228.....	37	45	55	47	9,277....	31	34	21	35
9,229.	24	25	18	19					
					Total..	214	238	230	222

showed that the locus of Delta was extraordinarily close to that of Star. Furthermore, there were 452 recombinations of "Star" and black in a total of 904 flies; or exactly 50 per cent of recombination, while about 40 is the expectation for Star and black. These two unusual circumstances—abnormally close linkage of "Star" and Delta, abnormally free linkage of "Star" and black—suggested that Delta might be due to a deficiency.

DELTA × ARISTITA.

To test the deficiency hypothesis, Delta was crossed to aristita, a recessive whose locus is about a unit to the right of that of Star, and it was observed that in F_1 none of the Deltas were aristita, so that the deficiency could not be very extensive. One of the F_1 "Star" Delta females was crossed again to aristita, with the surprising result that half of the flies were recombinations for "Star" and aristita, while one per cent is the expectation (9316; "S" Δ 43, ar 45, "S" Δ ar 44, + 45). Apparently the discrepancy was in some way connected with the "Star." A close examination of the "Star" was then made, and it was found to differ slightly but characteristically from true Star. In fact, all the evidence could be explained on the assumption that the original Delta male had not carried Star at all, but that the eye character was produced by the same gene that produced the Delta, which gene gives independent assortment with black and with aristita and is therefore probably not even in the second-chromosome.

DELTA × DICHLETE HAIRLESS.

The gene, not having been found in the second-chromosome, was presumably in the third, and accordingly an experiment was started at once to determine its location within the third-chromosome by means of a back-cross with

TABLE 111.—*P_1, Dichæte Hairless × Delta; B. C., F_1 D Δ H ♀ × wild ♂.*

Feb. 27, 1919.	D H	Δ	D Δ	H	D	Δ H	D Δ H	+
9,443.....	73	62	27	20	2	2	..	1
9,461.....	71	61	20	24	2	8	.	.
9,533.....	111	103	26	21	2	7	1	1
Total.....	255	226	73	65	6	17	1	2

the two dominants Dichaete and Hairless (table 111). The locus of Delta was found to be in the third chromosome between those of Dichaete and of Hairless, and about 4 units to the left of Hairless.

DELTA + PINK BAND

The other third chromosome of the Dichaete Hairless stock had carried pink and band. The not Dichaete not Hairless Delta F_1 females from the above F_1 were back crossed by pink band males (table 112). The locus of

TABLE 112.—*F_{11}, pink band + Delta; B. C., F_1 ♀ × p bu ♂*

Feb. 24, 1919	p bu	+	p +	+ bu	p + bu	+ + bu	+
9,416	58	55	14	14	5	4	1
9,419	49	49	12	13	4	5	1
Total	107	104	26	27	9	9	2

band is about 3 units to the right of Hairless and the Delta band recombination per cent was 6.8, agreeing with the location found by the Dichaete Hairless cross.

DICHAETE DELTA + PEPPER

It was anticipated that Delta would be a useful character because of its dominance and location. To determine its location with respect to the loci

TABLE 113.—*C D Δ*

April 23, 1919	+	D	D	+	+	D	+	D	+	D	+	D	D	+	Δ
	+	Δ	Δ	+	Δ	+	Δ	+	+	Δ	+	D	Δ	+	Δ
	+			+		+		+		+		+	+		
													+		
9,717....	14	14	2	4	7	7	2	4	6	1	21	17			
9,718....	11	19		2	6		2	1	1		1	1			
9,739	38	47	4	7	4	19	9	6	2	2	11	11	1	1	
9,735	44	33	2	3	4	10	3	4	1		12	7	1	1	
Total	127	113	8	16	21	36	16	15	10	7	51	41	2	2	
	+		+		D	+	+	D	D	Δ	D	+	+	+	
Cor.	D	+	D	+	D	+	+	D	D	Δ	D	+	+	Δ	
rigues	+	+	Δ	+	Δ	+	+	+	+	+	Δ	+	+	+	
	+		+		+			Δ			+	+			
9,717....		1		1	2	1	2	2			1			3	
9,718														1	
9,739....	2	3	1	1	1	1	1	1	1			1	1		
9,735	1	2		1				1		1	2				
Total	3	6	1	2	3	2	1	4	1	1	3	1	1	4	

ebony and spineless and to see whether its linkage relations were normal (no cross-over modifier present) a rather complex back-cross was made, namely, Dichæte Delta by sepia spineless sooty rough (table 143). The spineless Delta recombination value was 9.8, and the Delta sooty 4.2, both in conformity with expectation. The sepia Dichæte and Dichæte spineless were both low, but still probably normal.

DELTA × HAIRLESS SOOTY.

A Delta by Hairless sooty back-cross gave 2.9 and 1.6 as Delta Hairless and Hairless sooty recombination per cents (table 144).

TABLE 144.—P_1, *Hairless sooty* ♂ × *Delta* ♀; *B. C.*, F_1 Δ *H* ♀ × e^s ♂.

Dec. 18, 1920.	Δ	H e^s	Δ H e^s	+	Δ e^s	H
12,256.....	105	112	2	4	3	2
12,257.....	118	122	4	3	..	..
12,258.....	155	113	7	6	3	3
12,259.....	125	144	3	5	2	3
12,260.....	88	87	..	2	3	1
Total.....	591	578	16	20	11	9

DELTA LETHAL WHEN HOMOZYGOUS.

That Delta is lethal when homozygous was proved by crossing Delta to CIII IIIIa and mating together the Delta flies. These matings gave only Delta flies. These, inbred, have produced a pure-breeding stock of the mutation. Likewise, a Delta sooty was crossed to CIII IIIIa, and the Delta offspring inbred. No sooty appeared in the succeeding generations, showing that only heterozygous Deltas were present. A Delta Hairless sooty cardinal stock is carried along as an unselected stock balanced against CIII IIIIa.

DESCRIPTION OF DELTA.

The mutant called Delta is characterized by many differences affecting all parts of the body. The general body-color is distinctly darker than normal. The eyes are smaller and rough like Star, with strong dark hairs. The bristles are fairly stout and slightly curved. The legs are short. The wings are darker, smaller and somewhat pointed, and usually spread apart. The veins are thickened, especially at the tips of the wings.

NEUTRALIZATION OF DELTA BY HAIRLESS.

The general body-color of Hairless is distinctly lighter and yellower than normal. The eyes are large, smooth and short-haired. The hairs on the thorax are delicate and less numerous than normal. The bristles are missing, or are somewhat slender and long. The veins are weak, especially at the tip of the wing, where they often fail completely. The fourth and fifth longitudinals,

which are the ones that are the most delta-like in Delta are the weakest and shortest in Hairless. When Delta was crossed to Hairless it was found that the Delta Hairless double heterozygote was nearly wild-type in appearance—in color, eyes, hairs, bristles, and venation. The Hairless flies could still be distinguished by the absence of the post-verticals from behind the ocelli, and by the presence of a few empty bristle-bases on the abdomen. In making such classifications it was more convenient to separate out all the Hairless flies first and then to classify for Delta. The Delta Hairless flies were distinguished from the Hairless by the fact that the longitudinal veins—especially the fifth—always reached the margin and sometimes were faintly Delta. There are other minor points of distinction, which help in case of doubt, so that it is believed that Delta and Hairless may be used in the same experiment with complete certainty.

GENIC BALANCE.

It is possible and even probable that both Delta and Hairless are deficiencies. If so, the differences in the characters, and the fact of their neutralization helps materially in realizing that each normal character is the result of a balance between many genes; the deficiency for the section involved in Hairless removed genes whose net action may be called an intensification, so that the Hairless characters are relatively less pronounced than normal. But the neighboring section, involved in the Delta-deficiency, normally contained genes whose net action is a weakening of certain characters. The removal of those weakeners by deficiency produces a corresponding complex of intensified characters called the mutant type Delta. The wild-type fly is in balance; the Delta Hairless fly is in approximately that same balance, since the loss of "positive" genes (Hairless) is neutralized by the loss of "negative" genes (Delta).

SPINELESS SOOTY × DELTA.

Some additional evidence on the location of Delta has been secured by Carl E. Karns, who carried out a spineless sooty × Delta back-cross (table 145).

TABLE 145.—*Spineless sooty × Delta, Back-cross* (*Carl E. Karns*).

May 1, 1922.	ss e^s	Δ	ss Δ	e^s	ss	Δ e^s
1	70	72	5	6	2	4
2	58	57	5	4	3	4
3	66	68	5	6	3	3
4	49	50	4	4	2	3
5	63	65	4	6	3	4
6	73	72	5	6	4	3
Total	379	384	28	32	17	21

There was 7.0 per cent of recombination for spineless and Delta and 4.4 for Delta and sooty, which values agree well with the standard values.

HAIRY (h).

(Figure 30.)

ORIGIN OF HAIRY.

In locating the sex-linked recessive "singed" by a back-cross with the inflated forked Bar stock, Dr. O. L. Mohr found that many of the back-cross

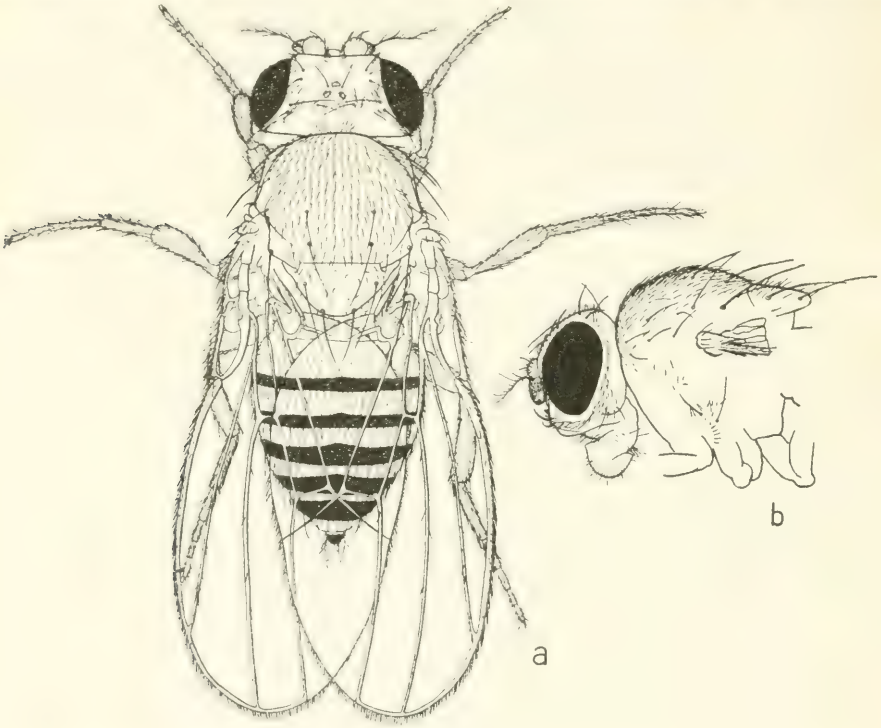


FIG. 30.—Hairy.

flies possessed hairs on the scutellum where normally no hairs are present (C 906, December 11, 1918). From these flies a "hairy" stock was obtained that bred true.

DESCRIPTION OF HAIRY.

Besides the hairs on the scutellum, other extra hairs were found to be present along the veins of the wings, on the sides of the thorax, and on the top of the head. These hairs are small, of the same size as the microchæte, but are distinct, so that the mutant character is classifiable with certainty and with fair speed. Hairy was immediately recognized as exceptionally promising because it would not be masked by other mutants, or interfere with the classification of any of them.

HAIRY × STAR DICHÆTE.

Since hairy was obviously an autosomal recessive, it was crossed by Mohr to Star Dichæte; and F_1 Star Dichæte males were back-crossed to hairy females.

The distribution of the characters proved that hairy is located in the third-chromosome. Female back-crosses of Star Dichæte by hairy were then made with the results given in total in table 146. There was 14.9 per cent of recombination for hairy and Dichæte.

TABLE 146.—*Summary of hairy × Star Dichæte ♀ B. C. (O. L. Mohr).*

March 25, 1919.	h	D	h D	+	S h	S D	S h	D S
M1167.....	133	186	28	22	130	166	31	27

The above experiments were carried out by Mohr during the last days of his stay at the Columbia laboratory. Before his departure for Norway, Mohr turned in some of his stocks of hairy, so that the mutant could be located and incorporated into useful multiple stocks without delay.

HAIRY × DICHÆTE; HAIRY × DICHÆTE HAIRLESS.

Two hairy by Dichæte back-cross cultures raised by Bridges gave respectively 16 and 8 per cent of recombination (table 147). From the "high"

TABLE 147.—*Hairy × Dichæte, ♀ B. C.*

Jan. 24, 1919.	h	D	h D	+	Total	Recomb. %
9,313.....	93	123	18	23	257	15.9
9,346.....	87	78	6	8	179	7.8
Total.....	180	201	24	31	436	12.6

culture a hairy female was out-crossed to a Dichæte Hairless male; and the back-cross results (table 148) showed that the locus of hairy was to the left

TABLE 148.—*P_1, hairy × Dichæte Hairless; B. C., F_1 D H ♀ × h ♂.*

Feb. 22, 1919.	h	D H	h D H	+	h H	D	h D	H
9,405.....	73	52	12	16	34	28	5	5
9,415.....	71	54	17	18	42	34	5	7
9,440.....	57	51	10	12	22	33	10	5
9,527.....	14	13	3	4	8	5	1	..
9,529.....	4	11	3	3	1	2	..	1
9,530.....	34	38	9	10	12	18	2	5
Total.....	253	219	54	63	119	120	23	23

of those of Dichæte and Hairless. The hairy Dichæte recombination per cent was 18.6. This value was greater than that given by sepia and Dichæte. Hairy

might therefore be situated to the left of sepia, a very favorable location, if correct.

PARALLEL BACK-CROSSES—HAIRY DICHÆTE AND SEPIA DICHÆTE.

The next points to be determined were the order and the recombination percentage of the loci sepia and hairy. The indications were that hairy was to the left of sepia; but the hairy Dichæte recombination value had been observed to be variable, and the experiments with sepia Dichæte had shown that this was a region subject to considerable variation. Therefore, only values obtained from the same experiment could be safely compared in finding the relative order of genes in this region by reference to a common base. The method of the "parallel" back-cross is used in such cases; that is, sister flies are used for the determinations, and since the two lots of sisters have the same constitutions, the results are comparable. In the present case a hairy Dichæte male was crossed to a female carrying sepia (se ss e^s ro). Dichæte daughters were tested by back-crossing to hairy males; other Dichæte daughters were tested by back-crossing to sepia (se ss e^s ro) males. One lot of offspring (table 149) gave a hairy Dichæte recombination percentage of 13.4, while

TABLE 149.—P_1, *hairy Dichæte* × *"seple"* (*se ss* e^s *ro*); *B. C.*, F_1 *D* ♀ *h* ♂.

April 11, 1919.	h D	+	h	D	Continued.	h D	+	h	D
9,661	210	246	31	43	9,671	279	307	39	43
9,662	204	188	35	32	9,672	269	282	34	50
9,663	197	189	30	26	9,681	218	225	20	29
9,664	119	135	28	15	9,682	125	102	21	16
9,670	194	233	43	40					
					Total	1,815	1,907	281	294

the other lot (table 150) gave a sepia Dichæte recombination percentage of 14.9. The differences between these values, 1.5 per cent, was so slight as to

TABLE 150.—P_1, *hairy Dichæte* × *"seple"* (*se ss* e^s *ro*); *B. C.*, F_1 *D* ♀ × *"seple"* ♂.

April 11, 1919.	se D ss e^s ro		se D ss e^s ro		se D ss e^s ro		se D ss e^s ro		se D ss e^s ro		se D ss + e^s ro		se D ss e^s ro		se D ss e^s ro		se D ss e^s ro		se D ss e^s ro		se D ss e^s ro		se D ss e^s ro		e^s ro
9,665	74	91	15	20	21	27	19	18	30	33	1	..	4	3	3	2	2	3	5	4	1	2	..	..	1	1	2
9,666	56	73	12	11	10	17	10	17	15	25	1	2	1	5	3	2	1	..	3	2	..	1	..	..	1	..	..
9,667	117	116	27	29	25	31	27	26	34	39	3	4	4	5	3	7	3	1	9	7	1	..	1	..	..	..	..
9,668	113	119	23	24	10	23	21	17	42	39	5	..	3	4	2	8	2	..	3	1	2	..	..	1	1	..	..
9,772	65	76	16	11	4	4	10	10	19	20	1	2	1	3	1	2	..	..	..	3	..	1	..	..	..	..	..
Total	425	475	93	95	70	102	87	88	140	156	11	8	13	20	12	21	8	4	20	17	4	4	1	1	3	1	2

be unreliable as proof of the order of the genes sepia and hairy; but nevertheless the attempt to secure a double recessive had to be made first on the basis of this indication.

SEPIA HAIRY × DICHÆTE.

If the locus of hairy is to the right of that of sepia, then some of the se D flies of table 150, that represent crossing-over between sepia and Dichæte, resulted from crossing-over in the left-most part of that section, and should contain a chromosome that carries the sepia part of the other parental chromosome. To know whether a particular sepia Dichæte fly has received the hairy gene, they were out-crossed to hairy. Of the 15 flies tested, one gave hairy offspring; and their hairy Dichæte offspring were known to carry sepia

TABLE 151.—*P_1, sepia hairy × Dichæte; B. C., F_1 D ♀ × se h ♂.*

July 23, 1919.	se h	D	se D h	se h D +	Continued.	se h	D	se D h	se h D +
10,239.....	169	165	1 3	20 19	10,267.....	135	162	.. ..	25 25
10,240.....	145	138	.. ..	19 15	10,268.....	94	91	.. 1	5 6
10,241.....	151	148	.. 1	13 16	10,269.....	166	164	1 1	20 16
10,242.....	163	136	1 3	23 28	10,270.....	154	158	.. 1	19 14
10,243.....	133	116	1 1	17 12	10,271.....	120	124	1 ..	10 15
10,244.....	146	105	.. 1	8 18					
					Total...	1,576	1,507	5 12	179 184

hairy and Dichæte in one chromosome and hairy in the other. By breeding from such flies, a sepia hairy stock was secured. (This stock was at the same time sooty.) A sepia hairy × Dichæte back-cross (table 151) was carried out. It was found that there was 0.5 per cent of recombination of sepia and hairy.

SEPIA HAIRY SOOTY BACK-CROSS.

A sepia hairy sooty back-cross was carried out in getting Star-intensifier hairy stock (table 127). The percentage of recombination was 0.6, which is in agreement with the 0.5 of the sepia hairy × Dichæte back-cross above.

HAIRY × DICHÆTE; HAIRY × SCARLET.

In preparing the multiple stocks with which to carry out experiments on the effect of age on crossing-over in the third-chromosome, roughoid hairy × scarlet F_1 females were tested by hairy scarlet males. The hairy scarlet recombination per cent was 25.0 (table 152) which is significantly higher than the standard

TABLE 152.—*P_1 [roughoid] hairy × scarlet; B. C., F_1 + ♀ × h st ♂.*

Aug. 28, 1920	h	st	h st	+
11,943.....	92	90	35	31
11,944.....	143	147	50	47
11,945.....	150	150	50	47
11,946.....	149	129	38	38
Total.....	534	516	173	163

per cent of 16.5. A hairy × Dichæte back-cross gave a normal value of 13.9 (table 153).

TABLE 153.—*P_1, hairy × Dichæte; B. C., F_1 D ♀ × h ♂.*

Aug. 7, 1920	h	D	h D	+
11,878	142	144	34	19
11,879	248	271	41	59
11,880	223	237	29	38
Total	613	652	104	116

EVALUATION OF HAIRY.

Hairy is located 0.5 unit to the right of sepia, and is more generally useful than sepia. These two loci become alternates in linkage experiments, with the preference given to hairy, which is now part of the standard III–chromosome multiple recessive. It is possible that the hairy stocks carry a linkage modifier that makes crossing-over in this region abnormally high. It was suspected that a like condition obtains for the roughoid stocks; but in neither case is the situation worked out.

MINUTE (M).

(Figure 31.)

ORIGIN OF MINUTE.

In locating the recessive "hairy" by means of a hairy × Dichæte back-cross (table 147), Bridges found among the offspring of one pair a single hairy male whose bristles were notably shorter and weaker than normal (9346, February 8, 1919). On account of the minuteness of the bristles, this mutant has been christened "Minute." This hairy "Minute-bristle" male was out-crossed to black, the hairy character serving as a control for the third-chromosome and black for the second. In F_1 no Minute-bristle flies were observed. Several wild-type F_1 pairs were mated in order to find the F_2 relation of the new mutant to black and to hairy. No Minute-bristle offspring appeared in these F_2 cultures; but instead, a recessive lethal was present in about a quarter of the cultures (table 163), the remainder of the cultures giving simple 9:3:3:1 ratios for black and hairy. Fortunately, the F_1 culture (9402) had been put aside in reserve, and when it was examined it was found to contain a large proportion of Minute-bristled flies. These were bred together, and in the next generation it was observed that the first counts from the culture-bottles consisted entirely of normal bristled flies, but that on the fourth or fifth day a few Minute-bristle flies began to appear. The relative number of Minutes then rapidly increased until

FIG. 31.
Minute-bristles.

in later counts most of the flies were Minutes. The first Minutes to appear were as extreme as the later ones, so that it was not probable that the first hatched flies that appeared to be normals were genetically Minutes. The real situation is that Minute-bristle is a dominant character and that the mutant flies are much delayed in emergence.

DESCRIPTION OF MINUTE.

The most obvious characteristic of Minute is that all the bristles of the fly, especially those on the thorax, the scutellum, and the head, are much more slender and considerably shorter than normal. These bristles have the same positions and directions as those in the wild fly. None are missing. In most Minute flies the minuteness of the bristles is the only departure from normal that is readily seen. But there is a tendency for the flies to be paler in general color, to show a darker trident pattern, to be smaller in size and to have blunter and dull-colored wings, characters present in other Minutes found later and also in Diminished flies. In the early cultures of Minute there were present three other characteristics that were very marked, namely, an "arcing" of the wing, a reduction in the size of the arista, and a fusion of the basal joints of the two fore-legs. It seems probable that these features were due to special modifiers, since they are no longer observed. However, similar reduction of the arista is a normal but variable feature of certain other Minutes and of Diminished.

MINUTE × "PEPLE."

The third-chromosome recessive hairy was present with Minute in the stock cultures, and the distribution of hairy and Minute showed that Minute was probably in the third-chromosome, but far removed from hairy. Up to this time no exact work had been done with Minute, and indeed it was regarded as a rather poor character. But when Minute was out-crossed to "peple" (p^p ss e^s ro), the character came out clearly, and in numbers approximately equal to those of the wild-type (table 154). Both male and female back-crosses

TABLE 154.—*P_1, hairy Minute × "peple"; B. C., F_1 M ♂ × "peple" ♀.*

F_1; July 10, 1919	M	+
10,210	104	93
10,212	113	137
Total	217	230
B. C.; Aug. 2, 1919	**M**	**"peple"**
12,259	126	120
12,260	124	155
12,261	149	116
12,264	168	157
Total	567	548

were carried out. The male back-crosses (table 154) proved definitely that Minute is in the third-chromosome. The female back-crosses showed that the locus of Minute is somewhat to the right of that of rough; but the counts were discontinued, since spineless Minutes were not certainly distinguishable from simple spineless. In both back-crosses the number of Minutes was not behind that of the other characters, although the Minutes were again characteristically late in emerging.

MINUTE × "SEPLE," AND × "SEBLE."

Since the character Minute can not be accurately classified in flies that are at the same time spineless, a mating was carried out which used all the characters except spineless of the multiple "seple" (se ss e^s ro). Minute was crossed to seble (se bx e^s ro, and the F_1 Minute females were crossed to seple. In the back-cross progeny (table 155) neither spineless nor bithorax appeared, but

TABLE 155.— $\frac{se \quad [bx] \quad e^s \quad ro}{M}$ ♀ × *se* [*ss*] e^s *ro* ♂.

Sept. 22, 1919	se e^s ro	M	se	e^s ro	M	se e^s	ro	M	se e^s ro +	M	se ro	e^s	M	se	e^s ro	M	se e^s	ro	M
10,501.....	34	35	20	22		4	8		7	3	3	6		1	4		.	1	
10,502.....	58	54	34	24		12	16		9	3	5	7		4	1		.	.	
10,503.....	22	23	12	14		6	11		3	3	2	2		2	2		.	.	
10,696.....	53	51	36	38		16	21		12	11	9	9		3	4		.	1	
10,697.....	63	51	31	24		21	15		9	8	8	4		5	2		.	1	
10,698.....	31	35	32	25		11	10		6	6	7	6		4	.		.	.	
Total.....	261	249	165	147		70	81		46	34	34	34		19	13		.	3	

sepia, sooty, rough, and Minute could be distinguished. For the next generation the Minute females (Minute over seple) were crossed to seble (table 156),

TABLE 156.— $\frac{se \quad [ss] \quad e^s \quad ro}{M}$ ♀ × *se* [*bx*] e^s *ro* ♂.

Oct. 18, 1919.	se e^s ro	M	se	e^s ro	M	se e^s	ro	M	se e^s ro +	M	se ro	e^s	M	se	e^s ro	M	se e^s	ro	M	se	e^s ro	M
10,581..	26	37	33	8		12	15		4	8	9	8		4	4		..	1		..	..	
10,582..	33	31	19	17		15	10		5	7	5	3		4	2		..	..		..	..	
10,585..	36	33	31	35		7	7		4	2	5	6		3	2		..	..		..	..	
10,586..	41	35	27	21		13	12		2	5	2	7		4	1		..	..		..	..	
10,590..	45	35	26	36		9	12		5	2	6	6		3	2		1	..		1	..	
10,830..	49	48	39	37		14	16		7	9	9	3		4	..		..	..		..	..	
10,831..	54	58	30	28		19	15		7	10	3	4		2	1		..	..		..	..	
10,832..	39	41	32	30		19	15		7	6	6	4		4	4		..	1		..	..	
Total.	323	318	237	212		108	107		41	49	45	41		28	16		1	2		1	..	

and so on for alternative generations. This procedure was used to see whether or not the same recombination percentages would occur with the two stocks seple and seble. When the χ^2 test was applied it was found that the results from the two stocks were nearly identical, as they had appeared to be upon simple inspection (table 156). The locus of Minute was found to be 9.5 units to the right of rough.

SEPIA MINUTE × DICHÆTE SOOTY ROUGH.

The character Minute had appeared in a fly that was also hairy, and work with hairy had shown that the hairy stock was giving somewhat different recombination percentages from those hitherto recognized as standard. The locus of the supposed modifier responsible for this change lies somewhere to the left of hairy. To eliminate the modifier, the left end of the Minute chromosome was replaced by a new end from the sepia white-ocelli stock; and a sepia Minute by a Dichæte sooty rough back-cross test was made (table 157).

TABLE 157.— $\frac{se}{D \quad e^s} - \frac{M}{ro}$ ♀ × "*seple*" ♂.

c.18, 19.	se M	D e^s ro	se D e^s ro	M	se e^s ro	D M	se ro	D e^s M	se e^s ro	D M	se D e^s M	e^s ro	se D e^s M	ro	se D e^s ro M	+	se e^s ro	D ro	se e^s ro M	D	se ro M	se D e^s ro M	se D
,033	64	56	10	8	37	18	23	17	7	6	3	3	2	1	1	2	3	2	3	6	.	.. ..	..
,034	46	47	8	7	20	16	13	20	6	4	2	1	4	..	3	1	5	2	2	2	..	.. 1	..
,035	45	60	9	5	26	17	12	14	6	3	3	2	4	4	3	..	2	2	5	1		1 ..	1
,036	27	33	3	9	10	5	7	6	4	1	2	1	2	1	1	..	.	.		1	1	.. ..	..
,043	51	66	8	8	22	32	14	19	5	8	1	2		2	1	..	4	3	1	2		. 1	1
,044	51	63	11	16	14	24	24	13	11	6	6	2	1	2	2	2	.	4	..	2	..	. ..	..
,045	65	62	13	12	23	20	13	17	5	6	5	4	3	3	..	1	3	1	1		..	.. ..	..
,046	64	50	6	9	31	21	17	21	13	6	2	3	1	2	3	2	4	3		1	1	. ..	..
,059	44	36	7	5	28	12	21	10	5	3	3	2	..	3	.	4	1	1				.. ..	..
,062	55	56	11	10	34	18	21	7	14	8	4	1	3	1	1	1	1	4	2	2		1 ..	..
,063	61	57	8	3	29	24	18	9	14	8	5		..	2	.	1		1	..	2	1	. ..	..
,064	39	65	7	6	24	17	15	13	12	2	.	2	1	2	2	..	2	4	.	2	..	1	1
,065	29	42	8	6	16	17	20	6	8	1	1	1	.	2	2	1	2	2	1	2		. ..	..
,066	48	47	8	9	26	16	11	14	12	4	1	1	1	5	1	1	2	1	3	.		.. ..	..
,067	46	49	5	10	26	17	19	13	5	1	3	1	..	2	.	3		2	3	2	..	1 ..	..
	735	789	122	123	366	274	248	199	127	67	41	26	22	32	20	19	29	32	21	25	3	4 2	3

The new values were not materially different from the former, or from the standard values. The rough Minute recombination per cent was 8.6, which is in close agreement with the 9.5 of the former experiment.

The location of Minute at about 9 units to the right of rough gave a new right end to the third-chromosome; and since the character is not a poor one in any essential respect, it promised a new means of attack on several problems, such as the effects of CIII and point of attachment of "Pale" translocation.

MINUTE A LETHAL WHEN HOMOZYGOUS.

In carrying on the stock by breeding Minutes by Minutes, the percentage of Minute did not increase appreciably, which is a peculiarity of such dominants as Beaded, Truncate, Dichæte, etc., that are known to be lethal when

homozygous. In order to secure more valid proof of the death of Minutes when homozygous, an F_2 was run in which the Minute entered with a closely linked recessive. Thus, a sepia sooty rough Minute male was crossed to a wild female, and about 15 pairs of the F_1 Minute by Minute were mated. This large number of pairs was started because a majority of the Minute females of previous tests had failed to produce offspring. Only 5 of these 15 gave offspring (table 158). The ratio of Minute to not-Minute in F_2 was

TABLE 158.—*P_1, sepia sooty rough Minute ♂ × wild ♀; F_2, F_1 M ♀ × F_1 M ♂.*

Dec. 26, 1919.	+	M	se M	se e^s ro M	e^s ro M
11,080.....	145	214	41	1	1
11,081.....	152	216	28	1	.....
11,100.....	54	115	29	.....	1
11,101.....	18	33	10	.....	.....
11,103.....	45	80	20	.....	.....
Total...	414	658	128	2	2

790 : 414; that is, 66 per cent of the flies were Minute where 67 per cent are expected if homozygous Minute is lethal. Only 4 flies that were sooty or rough appeared, while the expectation on the not-lethal basis is that a third of the Minutes would be also sooty. These results furnish complete proof that homozygous Minute is lethal.

THE PRESENCE OF CIIIM.

Among the F_2 offspring of a preceding cross there were abnormally few flies representing crossing-over between Minute and the various loci to the left of Minute. The percentages of recombination calculated from the above experiment are: se e^s = 32.1, e^s ro = 0.0, ro M = 1.0. The standard values are respectively 31, 20, and 9. It is clear that something was present that prevented nearly all crossing-over in the region to the right of sooty while it was not affecting greatly the net amount in the section between sepia and sooty. There was no modifier in the Minute ancestry that would account for the result, and the source of the cross-over modifier, temporarily called CIIIM, is to be sought in the wild (New Hampshire) stock to which the Minute was crossed. (For further account of CIIIM see p. 220ff.)

ROUGHOID × MINUTE.

Since roughoid and Minute were the left and right ends respectively of the third-chromosome map, and were approximately 95 units of map distance apart, it became a matter of some interest to see whether in this case the percentage of recombination would exceed 50, there being no cases known in which the percentage of recombination does exceed 50. Accordingly, a roughoid female was crossed to Minute male, and F_1 Minute females were back-crossed

to roughoid males (table 159). The percentage of recombination is 45.8, which was farther below 50 than was expected.

TABLE 159.—*P_1, roughoid ♀ × Minute ♂; B. C., F_1 M ♀ × ru ♂.*

Apr. 5, 1920.	ru	M	ru M	+
11,656	82	64	71	70
11,657	44	35	41	57
11,658	75	56	40	50
11,723	52	56	25	38
Total	253	211	177	215

THE MINUTE EPIDEMIC.

Shortly after the discovery of Minute, Bridges found a second Minute that agreed with the first in bristles, wings, aristæ, and coloring, in dominance, and in markedly delayed emergence. This mutant (Minute-b) proved to be at 85 in the second chromosome, that is, in the long gap between curved and arc. In the six months following the discovery of Minute-b, no less than 25 Minute-bristle characters were found, and within a year this number had increased to about 50. Soon it was observed that one particular type of Minute was recurring, and in fact constituted the bulk of the cases. This type, called Diminished, differed slightly in its somatic characters from the others, and was found to be the result of haploidy for the fourth-chromosome. The many occurrences of Diminished were due to the high frequency of primary non-disjunction of the fourth-chromosome. The other Minute-bristled mutants differed from Diminished and from each other mainly in the degree of development of the several common characteristics, and in their location in the chromosomes. Thus, Minute-d was found to depend on the simultaneous presence of two dominant genes, one in the second-chromosome near curved (72±), the other in the third-chromosome beyond rough (95±).

It is to be presumed that mutants of the Minute-bristle type were actually as frequent before as after the discovery of Minute. A few had been seen, but it was believed that they were mainly fluctuants, since they did not reappear in F_2. The reason for this is now understood, namely, because early F_1 flies were mated, and the F_1 culture thrown away before the Minutes emerged. It is probable that other dominant mutants have been lost through failure to recognize the fact of delayed emergence. A few recessive small-bristled mutants were found, and recovered in F_2, notably tiny-bristle.

EVALUATION OF MINUTE.

Since the discovery and location of Minute, another Minute (Minute-g) has been found that is still farther to the right than Minute, and which is as serviceable in its other features as the original. Also an excellent recessive eye-color, claret, has been located within a unit to the left of Minute; and claret can be used with spineless while Minute can not. The females of Minutes are very frequently sterile. This fact, together with the delayed emergence, limits to some extent the usefulness of the mutant. But on the other hand,

since the Minute character is readily separable from the wild-type, and is a dominant, it is a valuable addition to the III–chromosome characters.

ROUGHOID (ru).

(Figure 32.)

An account of the mutant roughoid has been given by L. C. Strong in the Biological Bulletin for January 1920. The following is a summary of that work and of some later experiments:

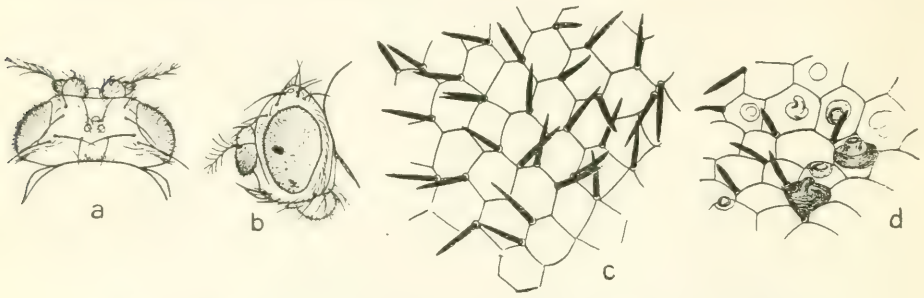


FIG. 32.—Roughoid eye. (*a* and *b*) top and side views; (*c*) enlarged view of facets and hairs; (*d*) enlarged view of black erupted facets.

The mutant "roughoid" was found by Sturtevant (February 14, 1919) to be present in considerable numbers among the F_2 offspring of a pair of wild flies collected at Columbia, South Carolina. Hence one of the wild flies must have carried the mutant gene.

The character of the eye is intermediate in roughness between Star and rough. It differs from other "roughs" in that it has a number of large black ommatidia scattered over the surface of the eye, though more frequent in the central part. These black ommatidia are still black when the roughoid eye is scarlet or scarlet pink. In ease of classification, viability, etc., roughoid is excellent.

The linkage experiments on the basis of which roughoid was located in the third-chromosome approximately 26 units to the left of sepia, which was the left-most of the then known third-chromosome loci, were carried out by Strong and are summarized in table 160.

TABLE 160.—*Summary of linkage data involving roughoid, from Strong, '20.*

ru × se	0	1	Total	Recomb. %
May 24, 1919	148	52	200	26.0
ru D × +	0	1		
July 10, 1919	123	69	192	35.9
ru se × +	0	1		
July 28, 1919	864	292	1156	25.2

ru se × D	0	1	2	1, 2		1	2
July 10, 1919	260	86	60	3	409	21.7	15.4

ru D H × se	0	1	2	3	1, 3	2, 3	1, 2, 3		1	2	3
May 24, 1919	136	66	45	29	16	5	6	303	29.0	18.4	18.4

ru se × D H	0	1	2	3	1,2	1, 3	2, 3	1, 2, 3		1	2	3
	317	119	82	90	3	37	24	5	677	24.2	16.7	2.31

The recombination values for se D in three of the experiments of Strong were 15.4, 18.4, and 16.7 respectively, which are the highest known for this section. It seems possible that the roughoid stock contained a linkage modifier that increased crossing-over in that section; or perhaps that some of the earlier low values were due to a linkage modifier that was preventing part of the crossing-over of that section.

In making up multiple stocks to test the effect of age upon crossing-over in the third-chromosome, Bridges made two sets of counts of recombinations in the roughoid sepia region (tables 161 and 162). The recombination per

TABLE 161.—P_1, *roughoid sepia*×*scarlet*; *B. C.*, F_1+♀×*ru se* ♂.

July 26, 1920.	ru se	+	ru	se
11,852	128	127	43	40
11,853	115	95	47	42
11,854	91	79	32	21
11,855	146	147	31	33
Total	480	448	153	136

TABLE 162.—P_1, *roughoid sepia*×*hairy*; *B. C.*, F_1+♀×*ru se* ♂.

July 26, 1920.	ru se	+	ru	se
11,856	116	115	50	62
11,857	145	146	54	47
11,858	137	130	46	43
11,859	150	153	54	60
11,860	151	166	40	47
11,862	168	162	79	81
Total	867	872	323	340

cents were respectively 24.6 and 27.6, both in sufficiently close agreement with the values found by Strong.

Roughoid is exceptionally valuable by reason of its position, which opens for investigation a section of the third-chromosome which is about 26 units long and whose existence makes the observed distribution of loci in the third-chromosome comprehensible.

COMPRESSED-DILAPIDATOR.

In working with the third-chromosome recessive compressed (see p. 193), Bridges found that the depressed original-combination class was abnormally low when compared with certain recombination classes; and that these original-combination compressed flies were relatively less robust (March 8, 1919, culture 9496, table 136). The analysis led to the conclusion that the compressed so far used had been modified specifically by a gene in the same chromosome with compressed and some 20 units to the right (at 68.5). As compared

with the simple compressed the modified compressed were pale in color, smaller in size, with weak legs, and thin ragged wings, and with markedly higher mortality. The "dilapidator" by itself produced little or no effect upon mortality or somatic characters.

LETHAL-IIIG (lIIIg).

In working with the mutation Minute, Bridges made some crosses of hairy Minute to wild, and raised several F_2 pairs. Two of the cultures from not-Minute F_1 pairs gave only a small percentage of hairy where 25 per cent was expected (March 20, 1919, cultures 9573, 9574, table 163). This was indica-

TABLE 163.—*P_1, Minute×hairy; F_2, F_1+♀×F_1+♂ (P_1 hairy het. for lIIIg).*

March 20, 1919.	+	h
9,573	88	4
9,574	70	9
9,709	255	6
9,748	328	11
Total	741	30

tive of a recessive lethal (lethal–IIIg) closely linked to hairy. In the next generation two other pairs of wild-type flies gave the same low percentage of hairy offspring (9709, 9748, table 163). The totals for the four cultures were 30 hairy and 741 wild-type flies. The hairy class is a simple recombination class ($rc = 30$), while the wild-type class is constituted from 3 original and 2 recombination classes ($3oc+2rc=741$). From these two equations the size of the corresponding original combination class is 227, and the percentage of recombination is 11.7. The stock was discarded without further experimentation.

PORT–B, A NON-MODIFIER OF EOSIN.

In an experiment in which the mutant Ski was tested as to whether it would dilute eosin as does "Pale," Bridges noticed that a maroon-like eye-color was present (table 164, September 11, 1919). The striking peculiarity

TABLE 164.—*P_1, eosin plexus speck ♀×[port-b] Ski ♂; F_2, F_1+♀×F_1 w^e ♂.*

Sept. 11, 1919.	+	port-b	px	sp	px sp	px sp port-b	w^e	w^e px	w^e sp	w^e px sp
10,447	76	28	1	..	22	10	134	1	4	24
10,448	73	25	1	1	11	6	75	1	2	28
Total	149	53	2	1	33	16	209	2	6	52

of this mutant, "port-b," was that, while it constituted approximately 25 per cent of the non-eosin flies, no difference in eye-color corresponding to the expected double-mutant type, eosin "port b," was detected in the eosin half of the progeny.

It seemed probable that "port-b" was sex-linked, and that it was so close to eosin that no double form occurred. But in that case "port-b" should have been present only in the males if it had entered from the grandfather, while in fact the color was equally distributed to the two sexes. Or, if it had entered from the grandmother the eosin "port-b" should then have been the large rather than the non-existing class. There were other considerations of such a kind that on the whole the evidence was conclusive that "port-b" was not a sex-linked mutant, either dominant or recessive.

The other alternative was that the double mutant, eosin port-b, occurs, but is indistinguishable somatically from simple eosin. This would be a case of specific non-modification quite the opposite of the "creams" reported by Bridges (J. E. Z., July, 1919) which were due to genes that gave no detectable effect except in the presence of eosin.

The presence of the second-chromosome recessives plexus and speck indicated the relations of the non-modifier to that chromosome. "Port-b" was distributed at random with respect to these mutants, which proves that the gene is not second-chromosomal. If port-b were second-chromosomal, the distribution of port-b and speck should have been in the form 2 : 1 : 1 : 0, or the port-b speck class should have been larger than the simple port-b class, neither of which conditions obtained.

This same mutant was found to be present in other crosses with Ski, under conditions that showed that it was a member of the third-chromosome, but without giving evidence as to its location within that chromosome. A stock of the mutant, put aside for special tests of this and other points, was lost without the tests having been carried out.

A similar and more striking case of a non-modifier of eosin is furnished by "brown," a second-chromosome eye-color that is as light in tone as eosin (female-type eosin), but which gives no cumulative effect with eosin, the double-mutant type being somatically indistinguishable from simple eosin.

WARPED (wp).

(Figure 33.)

ORIGIN AND DESCRIPTION OF WARPED-WING.

In an F_2 culture from a cross involving Minute and sepia white-ocelli, Bridges found that most of the sepia white-ocelli flies had wings that were held out from the sides at an angle of about 45°, and which were warped into a compound curve (10,764, November 15, 1919). The curvature of the "warped" wings is first upward, then downward, and again upward at the tip. The wings are also thin and somewhat crumpled, with a slightly waxy surface and a smoky color. They are narrower in shape and slightly smaller than the wild-type. The general body-color of warped flies is dark, with

the trident-pattern practically absent or submerged. The eyes are small and strongly convex. Other slight differences in aristæ, bristles, hairs, etc., exist;

FIG. 33.—Warped wing.

but in classification the most useful characteristics are those of wing position and texture.

SEPIA WARPED WHITE-OCELLI × HAIRLESS.

A rough analysis of the distribution of warped in the original culture showed that the mutant is recessive, in the third-chromosome, and probably situated between sepia and white-ocelli. The possession of the triple recessive made it easy to determine at once the position of warped. The somatic peculiarities of the warped wing made the simultaneous use of Dichæte open to question. Hairless was used instead in a back-cross with sepia warped white-ocelli (table 165).

TABLE 165.—*P_1, sepia warped white-ocelli × Hairless; B. C., F_1 H ♀ × se wp wo ♂.*

Dec. 13, 1919.	se wp wo		H		se H		wp wo		se wp H		wo		se wp		H wo		se wp		H wo		se wp		H + wo	
11,005	37	49			8	15			11	9			..	3			8	1			2	..		
11,006	114	108			37	26			24	30			10	11			7	7			..	1		
11,020	50	66			22	24			11	19			7	6			3	7			5	3	1	.
11,021	63	96			30	23			19	24			5	7			9	5			1	3	1	.
11,126	46	59			21	25			13	15			9	8			8	6			2	1		
11,127	97	118			32	22			29	24			10	9			6	13			2	3	1	.
11,128	84	81			19	22			28	18			9	7			7	7			3	5	.	1
Total	491	577			169	157			135	139			50	51			48	46			15	16	3	1

The locus of warped proved to be between those of sepia and Hairless, giving 23.9 per cent of recombination with the former and 19.7 with the latter. These data placed warped roughly in the group of mutants clustered about pink, probably in the region to the right in that group.

SEPIA WARPED × DEFORMED.

The relative positions of the loci in the group about pink is difficult to determine because of the labor involved in securing suitable combinations where the amount of crossing-over is slight, and also on account of the relatively large variability in crossing-over observed for that region. These loci will in time be related to pink as a base of reference. In this connection the dominant Deformed, situated 0.5 unit to the left of pink, is useful for making a tentative location of the genes of this group. Deformed was used in a back-cross with sepia warped (table 166); but with very unsatisfactory results,

TABLE 166.—*P_1, sepia warped × Deformed; B. C., F_1 Df ♀ × se wp ♂.*

Feb. 27, 1921.	se wp	+	se	wp	Df	se Df	Df wp	se Df wp
12,483.....	141	102	30	44	58	22	1	1
12,554.....	105	81	41	49	13	14	..	1
12,555.....	91	81	36	36	21	12	4	2
12,556.....	114	94	30	35	14	11	..	1
Total...	451	358	137	164	106	59	5	5

because the smallness of the eyes of warped made confusion with Deformed possible. Taken at their face value the data of table 166 would place warped to the right or to the left of Deformed by 5.7 units (Deformed flies only used). But in addition to the above difficulty in classification is the fact that sepia warped gave 28.4 per cent of recombination—a value quite aberrantly large. Considering all the data, the best value for the location of warped is perhaps within 4 units to the right of pink, or tentatively at 52±.

CARDINAL (cd).

(Plate 1, Figure 12.)

The mutant cardinal was discovered by Mr. H. H. Johnson, to whom we are indebted for the information in the following account: In the F_2 of a cross of vestigial to wild (Camp Jackson stock), about a quarter of the flies showed an eye-color very similar to the sex-linked recessive vermilion, or the third-chromosome recessive scarlet (November 24, 1919). This eye-color, called cardinal, resembles scarlet closely in its tone and in the cycle of change in color with age. However, cardinal approaches the wild-type more nearly than does scarlet in flies that have aged. The ocellar color of cardinal flies is white, even in the aged flies whose eye-color is identical with the red of the wild-type. The cardinal character was seen to be distributed at random with respect to sex and the second-chromosome recessive vestigial, and was therefore neither sex-linked nor second-chromosomal. A linkage experiment with Star Dichæte showed that cardinal is a member of the third group. Two small

cultures of Dichæte by cardinal, female back-cross, gave 40.5 per cent of recombination for Dichæte and cardinal (36/89). Four cultures of the back-

TABLE 167.—P_1, *cardinal* × *Dichæte Hairless*; *B. C.*, F_1 *D H* ♀ × *cd* ♂ (*H. H. Johnson*).

April 29, 1920.	DH	cd	D cd	H	DH cd +		D	H cd
Totals....	230	218	52	81	12	22	..	1

cross of cardinal by Dichæte Hairless (totals in table 167) showed that the locus of cardinal is to the right of that of Hairless by about 6 units.

DELTA HAIRLESS SOOTY × CARDINAL.

The mutant character cardinal had been present in the Camp Jackson wild stock, as Johnson found. Later, Bridges also found cardinal in the Camp Jackson stock, and determined its locus by means of a Delta Hairless × cardinal back-cross (table 168) as 6.5 to the right of Hairless. The combined data

TABLE 168.—P_1, *Delta Hairless* × *cardinal*; *B. C.*, F_1 Δ *H* ♀ × *cd* ♂.

Feb. 14, 1921.	Δ H	cd	Δ cd	H	Δ H	cd +
12,334.....	150	118	5	9	10	11
12,335.....	133	102	2	3	9	10
12,336.....	98	103	6	4	12	4
12,337.....	41	38	1	1	4	2
12,338.....	73	55	3	1	3	1
Total.....	495	416	17	18	38	28

indicate a locus 6.2 units to the right of Hairless or at 75.7. The locus of cardinal is thus very close to that of white-ocelli (76.2), and the two may be used as alternates. The order of these two loci must be determined by further experiment. The character cardinal is excellent in every respect, except that the flies must be classified when young, unless they are classified by means of the ocellar color.

SOOTY CARDINAL BACK-CROSS.

In attempting to find the serial order of cardinal and white-ocelli, some parallel back-crosses were started from a mating of sooty cardinal spineless

TABLE 169.—P_1, *sooty cardinal* × *ss wo*; *B. C.*, F_1 + ♀ × e^s *cd* ♂.

Aug. 12, 1922.	e^s cd	+	e^s	cd
13,228.....	199	169	9	10
13,229.....	147	174	8	12
Total.....	346	343	17	22

white-ocelli. The sooty white-ocelli back-crosses failed for lack of proper males to use in the back-cross, and only 2 of the sooty cardinal cultures (table 169) were kept as a sample. The cardinal classification was checked by the ocellar color.

CLARET (ca).

(Plate 1, Figure 9.)

ORIGIN OF CLARET.

In working with a strain of high non-disjunction that was producing triplo–X individuals, Bridges found that a pink-like eye-color was present unexpectedly (10965, December 12, 1919). This eye-color showed no linkage to the sex-linked characters eosin and forked that were present in the culture and was adjudged autosomal. A pure-breeding stock was secured and the sex-linked characters eliminated from it by selection.

CLARET × STAR DICHÆTE; CLARET × DICHÆTE HAIRLESS.

A claret × Star Dichæte male back-cross was started, and at the same time a claret by Dichæte Hairless and a claret by Star Lobe. The purpose of the Star Dichæte back-cross was to find which of the two latter experiments to discard. Claret was found to be in the third-chromosome, hence the claret × Dichæte Hairless back-cross only was continued (table 170). The results were

TABLE 170.—P_1, *Dichæte Hairless × claret; B. C., F_1 D H ♀ × ca ♂.*

Jan. 5, 1920.	D H	ca	D ca	H	D H ca	+	D	H ca
11,277.....	40	36	18	24	31	28	7	11
11,297.....	41	65	30	33	35	33	7	8
Total.....	81	101	48	57	66	61	14	19

surprising, for claret was apparently as far to the right of Hairless as Dichæte is to the left. That is, claret is to the right of rough.

HAIRLESS CLARET × MINUTE.

Not long before this the dominant "Minute" had been located some 9 units to the right of rough; and, from the results of the claret × Dichæte

TABLE 171.—P_1 *Hairless claret × Minute; B. C., F_1 H M ♀ × ca ♂.*

March 6, 1920.	H ca	M	H M	ca	H ca M	+
11,433.....	134	118	60	67	1	..
11,434.....	25	32	18	18	..	..
11,452.....	100	79	33	36	..	..
11,461.....	74	58	25	34	..	..
11,480.....	100	54	34	40	1	1
11,481.....	92	90	40	43	..	..
11,487.....	60	62	33	40	..	3
11,527.....	77	88	31	41	..	..
Total.....	662	581	274	319	2	4

Hairless back-cross, should be very close to the locus of claret. A Hairless claret female from the above back-cross was out-crossed to Minute; and several back-cross cultures were raised (table 171). The locus of claret was found to be very slightly to the left of that of Minute; the percentage of recombination being 0.3. The locus of claret is 100.7.

EVALUATION OF CLARET.

Claret is an eye-color very easy to classify, since it is a clear translucent pink, about the color of the sex-linked mutants ruby and garnet. Its viability seems excellent, and it is an acceptable alternate to Minute in all experiments in which it is not necessary to use the mutant pink.

CIIIM (CROSS-OVER VARIATION FROM MINUTE EXPERIMENT).

ORIGIN OF CIIIM.

To discover whether or not the dominant mutant "Minute" is lethal when homozygous, Bridges employed the method of raising F_2 cultures from crosses in which a recessive is present in the same chromosome with, and close to, the gene for the character to be tested. Thus, a sepia sooty rough Minute male was out-crossed to a wild female (from the New Hampshire stock of wild). F_2 was raised from 5 pairs of F_1 Minute flies (December 26, 1919, table 158, p. 210). Only 4 flies that were sooty or rough appeared, while the expectation on a non-lethal basis is that a third of the Minutes would be also sooty. This fact and the 2 M : 1 not-M ratio show that Minute is lethal when homozygous.

There were abnormally few flies that were sooty or rough, even assuming that Minute is lethal when homozygous. The percentages of recombination calculated from the above F_2 are: se e^s = 32.1, e^s ro = 0.0, ro M = 1.0, while the standard values are 34.3, 20.4 and 9.9 per cent respectively. That is, something was present that entirely suppressed crossing-over between sooty and rough, nearly suppressed that between rough and Minute, but that did not affect the crossing-over between sepia and sooty in such a way as to give an abnormal percentage of recombination. The result was accountable on the

TABLE 172.—P_1, *CIIIM × sepia sooty rough Minute, back-cross.*

Jan. 14, 1920.	se e^s ro M	+	se	e^s ro M
11,193	131	125	58	58
11,194	51	70	29	35
11,490	92	113	42	35
11,491	38	36	12	4
11,492	104	144	60	42
11,498	112	107	50	51
Total	528	595	251	225

assumption that a cross-over modifier was present that greatly reduced crossing-over throughout the region between Minute and some point to the left of sooty. The ancestry of the Minute was known, and no such modifier was

present, hence it seemed probable that the source of the modifier was the wild stock to which the Minute had been out-crossed.

SEPIA SOOTY ROUGH MINUTE × CIIIM, BACK-CROSS.

A more efficient method for finding the linkage relations in the presence of a linkage modifier is the back-cross. Accordingly, several Minute females from the above F_2 cultures were out-crossed to sepia bithorax sooty rough males. The results (table 172) showed 29.8 per cent of recombination for sepia and sooty, 0.0 for sooty rough, and 0.0 for rough Minute. These results are the same as those of the F_2, except that no crossing-over between rough and Minute is evident.

SEPIA SOOTY ROUGH × CIIIM, BACK-CROSS.

The F_2, and especially the back-cross results, showed that there was an inherited linkage modification present; and the pedigrees of the stock showed that if the locus of this modifier were in the third-chromosome the gene should be present in the not-Minute chromosome. Therefore several not-Minute daughters of the above back-cross were out-crossed to sepia spineless sooty rough males (table 173). There was 25.9 per cent of recombination for sepia

TABLE 173.—*CIIIM × sepia sooty rough, back-cross.*

Jan. 30, 1920.	se e^s ro	+	se	e^s ro
11,265	167	157	48	45
11,266	166	146	53	57
11,267	136	144	71	48
Total	469	447	172	150

sooty, and 0.0 for sooty rough, which means that the same cross-over modifier is present, and that it is a dominant located in the not-Minute third-chromosome, as assumed.

CIIIM × SEPIA SPINELESS SOOTY ROUGH, BACK-CROSS.

A CIIIM × "seple" back-cross (table 174) gave 27.6 per cent of recombination for sepia spineless, 0.0 for spineless sooty, and 0.1 for sooty rough.

TABLE 174.—*CIIIM × sepia spineless sooty rough, back-cross.*

Feb. 14, 1920.	se ss e^s ro	+	se	ss e^s ro	se e^s
11,326	98	129	55	38	.....
11,327	103	145	49	43	.....
11,339	65	86	30	23	1
Total	266	360	134	104	1

SPINELESS SOOTY ROUGH × CIIIM, BACK-CROSS.

The results so far were parallel to those expected for the original CIII, except that the effect of CIII ended rather abruptly at a point about 2 units to the right of pink, and the sepia sooty recombination per cent found for CIIIM seemed too high. The next point investigated was the crossing-over relation between CIIIM and pink. Flies heterozygous for CIIIM and for se ss e^s ro were out-crossed to p^p ss e^s ro males (table 175). The progeny contained no re-

TABLE 175.—*CIIIM over se ss e^s ro ♀ × p^p ss e^s ro ♂.*

Feb. 14, 1920.	ss e^s ro	+
11,324	230	263
11,325	150	208
11,337	104	142
11,338	78	98
Total	562	711

combinations for spineless sooty or for sooty rough. The wild-type offspring of that out-cross were females heterozygous for CIIIM and for p^p ss e^s ro that were desired to test for the relation of pink to CIIIM (table 176).

TABLE 176.—*CIIIM × peach spineless sooty rough, back-cross.*

Feb. 27, 1920.	p^p ss e^s ro	+	p^p	ss e^s ro	ro	ss e^s
11,391	147	192	..	..	.....	.....
11,392	100	124	1	..	.....	.....
11,393	111	152	5	1	.....	.....
11,394	157	203	1	..	.....	.....
11,396	205	182	..	1	.....	1
11,397	171	175	2	2	.....	.....
11,398	107	81	..	1	.....	.....
11,501	230	254	4	2	2	.....
11,503	82	90	..	..	.....	.....
11,530	158	209	2	..	.....	.....
11,532	159	186	..	1	.....	.....
11,533	127	159	..	..	.....	.....
11,534	148	195	..	1	.....	.....
Total	1,902	2,202	15	9	2	1

There was 0.6 per cent of recombination for pink and spineless (table 176), which means 0.6 per cent of recombination for pink and the end of the region sharply affected by CIIIM. There was no recombination for spineless sooty, and only 0.1 for sooty rough.

SEPIA CIIIM ROUGH × SPINELESS.

In culture 11339, table 174, occurred a double-recombination sepia rough fly. This gave an opportunity to make a test of the location of the gene CIIIM, by observing whether this gene were included in or excluded from the sepia rough

cross-over chromosome. The sepia rough male was out-crossed to a spineless white-ocelli female, with the production of 69 spineless and 67 wild-type offspring. The wild-type offspring of the above cross had received the cross-over chromosome, and the crossing-over relations in them were tested by out-crossing to se ss e^s ro males (table 177). There was 29.2 per cent of recombi-

TABLE 177.—*P_1 sepia* CHIIM *rough × spineless white-ocelli; B. C., F_1 + ♀ × se ss e^s ro* ♂.

March 17, 1920.	se ro	ss	se ss	ro	se	se ss ro
11,551	116	125	32	59		
11,552	118	105	55	45	1	1
Total	234	230	87	104	1	1

nation for sepia spineless and 0.3 per cent for spineless rough, the last value being evidence of the presence of CHIIM. The locus of CHIIM is therefore known to be to the left of that of rough, which narrows a little the range of possible positions.

SEPIA SPINELESS ROUGH × CHIIM.

In culture 11552, table 177, occurred a se ss ro fly, which was crossed to CHIIM, and two back-cross cultures raised (table 178). There was 25.5 per

TABLE 178.—*Sepia spineless rough (11,552) ×* CHIIM*; B. C., F_1 + ♀ × se ss e^s ro* ♂.

April 10, 1920.	se ss ro	+	se	ss ro
11,685	179	155	54	50
11,688	114	161	52	53
Total	293	316	106	103

cent of recombination for sepia spineless and 0.0 for spineless rough, both values being characteristic for heterozygous CHIIM. The cross-over that gave rise to the sepia spineless not–CHIIM rough fly had been to the right of CHIIM and to the left of rough; that is, CHIIM is to the left of rough.

SUMMARY OF RECOMBINATION DATA FOR HETEROZYGOUS CHIIM.

The recombination data from the eight separate experiments have been collected, and the percentage of recombination for each pair of loci, *e. g.*, se ss, or se M, has been calculated by the method described on page 20ff. These per cents are entered as "Total" recombination per cents, and the significance of each is proportional to the number of individuals involved (table 179). In calculating the map-distances that correspond to a set of percentages of

recombination, a given experiment should be used only once for any section of chromosome. Thus, in a three-point experiment ss e ro, the data for ss-e and for e-ro are called "primary," those for ss-ro, "secondary." The primary data for ss-e from the different experiments are combined to give the mean primary recombination per cent for that pair of loci.

The next step is normally to convert the mean primary recombination per cents into units of map-distance. This requires a study of double-recombination and of coincidence. In the present case no double-recombination individuals were observed for the loci within the region of reduced crossing-over; and the cross-over values may be taken as numerically the same as the recombination per cents. There is presumably double crossing-over in the region between sepia and the left end of the region of reduced crossing-over, that is, between sepia and a point just to the right of pink. But the amount of this double crossing-over is not determinable from the data in hand. It can not

TABLE 179.—*Summary of recombination data for* CHIM.

Loci.	Primary.		Secondary.		Total.	
	Per cent.	Numbers.	Per cent.	Numbers.	Per cent.	Numbers.
se-ss	27.4	2,340			27.4	2,340
se-e	28.6	3,240	28.4	4,107	28.5	7,349
se-ro			28.1	5,582	28.1	5,582
se-M			30.2	2,004	30.2	2,004
p-ss	0.6	4,131			0.6	4,131
p-e			0.6	4,131	0.6	4,131
p-ro			0.6	4,131	0.6	4,131
ss-e	0.0	6,269			0.0	6,269
ss-ro	0.1	1,475	0.1	6,269	0.1	7,744
e-ro	0.04	9,511			0.04	9,511
e-M			0.2	2,004	0.2	2,004
ro-M	0.2	2,004			0.2	2,004

be assumed that the amount of double crossing-over or the coincidence is the same as that normally present for this section; for it is observed that CHIM percentage of recombination is about 28.1, while the standard percentage of recombination for sepia pink is about 20.4 (see p. 13). An increase as great as this means that crossing-over relations in the region to the left of pink are also affected by CHIM. Perhaps some of the crossing-over that is prevented from occurring in the right limb of the chromosome is shifted over to the left limb, and a more thorough study of this phenomenon may help in understanding the mechanism of crossing-over.

For the present there is no advantage in making an assumption as to the probable coincidence for the sepia pink region in heterozygous CHIM, and the data may be left in the form of recombination per cents. "Standard-CHIM" recombination per cents can be calculated where several mean primary recombination per cents involve the same set of loci, *e. g.*, se ss = 27.4, se e = 28.6, ss e = 0.0. Since recombination for ss-e is zero or negligible, the se-e data can be converted into se-ss data by subtracting from it 0.0; that is, the se-ss and se-e data both represent recombination for sepia and the left end of the region

of reduced crossing-over, and they can be combined directly to give a standard-CIIIM recombination per cent of 28.1, which applies equally to se-ss and to se-e. Similarly, the ss-e, e-ro, and ss-ro data can be combined to give standard per cents of 0.0, 0.05, and 0.05 respectively. The standard per cents for p-ss, for ss-e, and for ro-M are given directly by the primary percentages.

COMPARISON OF CIIIM WITH CIII.

CIIIM was present in a wild stock (Camp Jackson, South Carolina) brought into the laboratory late in 1919. CIII is known to have been present in the inbred Beaded stock shortly after its discovery in 1910, and was therefore probably present in the wild stock (Woods Hole?, Massachusetts) from which Beaded arose. Because of this difference in time and locality, it does not seem probable that these two stocks represent one and the same mutation.

In heterozygous CIIIM there is apparently no crossing-over between spineless and ebony, and in heterozygous CIII there is only a very slight amount. The main difference seems to be that there is more crossing-over between ebony and rough in CIIIM than in CIII. It seems probable that there is less variation in the crossing-over and also a smaller total of crossing-over between pink and the reduced region for CIIIM than for CIII. Both these differences may well be due to a less definite boundary for the region of reduced crossing-over in the case of CIII than in that of CIIIM. If this point of change corresponds to the mid-point of the III–chromosome (see p. 29), then the CIIIM data offers a better index of this point than did CIII.

No information is on hand as to the linkage relations in homozygous CIIIM for comparison with the normal or increased crossing-over in homozygous CIII.

No tests of the possible allelomorphism of CIIIM and CIII have been undertaken as yet.

BITHORAX-B (bx-b).

(Figure 34.)

ORIGIN AND DESCRIPTION OF BITHORAX-B.

In working with the mutant compressed, Bridges found that a mutant closely resembling bithorax was present in nearly all the compressed flies of

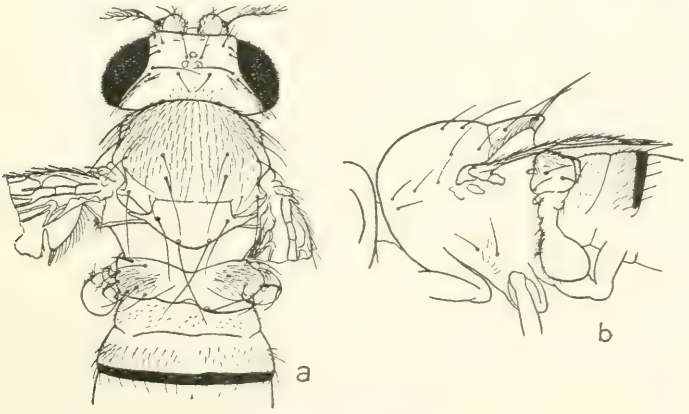


FIG. 34.—Bithorax-b.

certain cultures (11110, December 27, 1919). The mutant had balancers that were inflated, and that showed hairs, bristles, and venation characteristic of wings. The balancer-wings differed from those of bithorax in being inflated into rather balloon-like organs, instead of being flat and elongated. There was present also the haired and bristled representative of the meso-thorax inserted between the scutellum and the abdomen; but it was not so well-developed nor so clear as in the case of bithorax.

BITHORAX-B SOOTY ROUGH × HAIRLESS.

The fact that nearly all of the bithorax-b individuals were at the same time compressed showed that the locus of the mutant is in the third-chromosome. By a succession of matings, compressed was removed and sooty and rough were

TABLE 180.—*P_1, bithorax-b sooty rough × Hairless; B. C., F_1 H ♀ × bx-b e^s ro ♂.*

Jan. 26, '20.	bx-b e^s ro	H	bx-b H	es ro	bx-b	H e^s ro	bx-b e^s	H ro	bx-b H ro	e^s
11,250....	34	63	1	15	..	..	11	23	..	1
11,306....	105	131	11	15	..	4	28	36	..	..
11,317....	34	75	1	10	..	..	6	13	1	4
11,321....	67	41	3	7	..	..	27	14	..	..
11,334....	64	94	13	12	3	2	19	26	1	2
11,430....	57	111	10	16	4	1	17	27	..	1
11,468....	18	64	..	4	1	1	6	15	..	..
11,475....	95	142	11	16	2	1	26	34	..	1
Total.	474	721	50	95	10	9	140	188	2	9

combined with the bithorax-b character. A bithorax-b sooty rough by Hairless back-cross was then carried out (table 180). The locus of bithorax-b was thus shown to be to the left of Hairless by some 9 units.

BITHORAX-B × BITHORAX.

The above linkage result is nearly identical with that given by bithorax and Hairless; and since, both in somatic character and in location, the mutants were similar, the presumption was that the two were allelomorphs. This proved not to be the case, for crosses between bithorax and bithorax-b gave only wild-type F_1 progeny. This is the most striking case that we have met of the danger of judging allelomorphism from somatic characters or from linkage relations or from both.

PARALLEL BACK-CROSS BX × H, SS × H.

To determine roughly the special relations of the loci of bithorax and bithorax-b, parallel back-crosses of bx-b × H and of ss × H were carried out (tables 181 and 182). The spineless Hairless recombination per cent was 13.8, and the bithorax-b Hairless per cent was 12.8. The indications are, then, that the locus of bithorax-b is to the right of that of spineless by a unit, or within a unit of bithorax. The next step planned was to secure spineless

bithorax-b flies by testing cross-overs from the descendents of the parallel back-crosses. Since the indications were that bithorax-b is to the right of spineless, the spineless Hairless recombinations from table 182 should occasionally have resulted from crossing-overs between spineless and bithorax-b and should carry the desired spineless bithorax-b Hairless chromosome. About 20 such flies were tested, but none were the desired type. Instead of repeating

TABLE 181.—*P_1, bithorax-b Hairless × spineless white-ocelli; B. C., F_1 H ♀ × bx-b ♂.*

April 7, 1920.	bx-b H	+	bx-b	H
11,664	79	130	21	14
11,665	53	168	14	14
11,666	139	244	20	35
11,667	95	216	20	25
Total	366	758	75	88

this experiment, it would be more advantageous to cross bithorax-b to spineless glass. The spineless not-glass offspring should contain a much smaller proportion of "wasters" than in the case of the spineless Hairless cross-overs, since the reference points spineless and glass are much closer together. This method had been successful in getting the spineless bithorax double recessive.

TABLE 182.—*P_1, bithorax-b Hairless × spineless white-ocelli; B. C., F_1 H ♀ × ss wo ♂.*

April 7, 1920.	ss wo	H	ss H	wo	ss	H wo	ss H wo	+
11,668	142	180	18	35	18	10	1	..
11,669	105	116	21	18	11	13	..	1
11,670	144	164	25	28	9	8	..	..
Total	391	460	64	81	38	31	1	1

A still better method would be to cross spineless bithorax to bithorax-b and back-cross by spineless bithorax. All or nearly all of the few spineless not-bithorax cross-overs should have a spineless bithorax-b chromosome. With this stock, parallel back-crosses of spineless bithorax and spineless bithorax-b could be carried out. This work has not yet been done, so provisionally bithorax-b is mapped 1 unit to the right of spineless or at 59.5.

EVALUATION OF BITHORAX-B

The viability of the bithorax-b mutant stock is not good; and since this region is already well represented by spineless and bithorax, it is probably only in case of deficiency or duplication that bithorax-b would be valuable.

ROOF–C.

(Figure 35.)

In culture 11110, table 158 (p. 210), 20 of the flies entered as wild-type, 2 of the flies entered as sepia Minute, and 3 of the flies entered as Minute possessed wings that drooped at the outer margin (January 1, 1920). The distribution

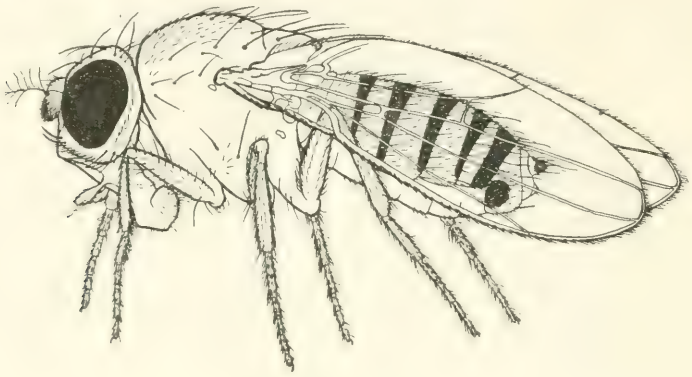


FIG. 35.—Roof-c.

of the character is such as to agree best with the assumption that "roof" is a third-chromosome dominant situated to the left of sepia. Unfortunately the stock was lost before these points could be demonstrated.

DWARF–B (dw-b).

(Figure 26*b*, page 182.)

ORIGIN OF DWARF-B.

In working with cultures of high non-disjunction that were giving extra–X females (2n+X), Bridges observed that in several cultures about a quarter of the flies of both sexes were very small and rather pale in body-color (11,258, February 5, 1920). By breeding together "dwarf-b" females and males a stock was established that bred true, but with considerable fluctuation in the size of the individuals.

DWARF-B × DWARF.

The mutant dwarf-b resembled the mutant dwarf very closely; but the fact that females of dwarf-b are fertile while females of dwarf are invariably sterile showed that they could not be identical. That they were not even allelomorphic was shown by the production of only wild-type offspring from the cross of dwarf-b by dwarf (11366).

DWARF-B × HAIRLESS.

A dwarf-b × Star Hairless male back-cross (11569) gave 252 dwarf-b and 235 Hairless offspring with no certain recombinations; which means that the gene is third-chromosomal. From this culture some female back-crosses

were made with the not-Star flies (table 183). There was 25.7 per cent of recombination.

TABLE 183.—P_1, *dwarf-b* × (*Star*) *Hairless; B. C., II* ♀ × *dw-b* ♂.

March 10, 1920.	dw-b	H	dw-b H	+
11,678	135	141	64	46
11,718	82	95	24	26
Total	217	246	88	72

DWARF-B × DICHÆTE HAIRLESS.

The classification of dwarf-b had not been very accurate in the dwarf-b × Hairless back-cross, and the same difficulty was experienced in the dwarf-b × Dichæte Hairless back-cross separations (table 184). In culture 12043 the

TABLE 184.—P_1, *dwarf-b* × *Dichæte Hairless; B. C., F*$_1$ *D H* ♀ × *dw-b* ♂.

Oct. 25, 1920.	dw-b	dw-b D H	dw-b H	dw-b D	D H	+	D	H
12,043	72	27	20	5	89	31	23	18

flies clearly dwarf-b were separated out first and then reclassified according to the characters Dichæte and Hairless. The locus is apparently quite far to the left of that of Dichæte. The dwarf-b Dichæte recombination per cent was 25.7, while the dwarf-b Hairless value was 37.7, which is not in agreement with the value found for the simple dwarf-b × Hairless back-cross. The classification of dwarf-b is so laborious and so uncertain that no more linkage experiments were undertaken. The locus may be placed at 12 ± by comparison with recombination per cents given with Dichæte by other mutants in the region to the left of sepia.

WEIGHT CURVE OF DWARF-B FLIES.

It was noticed that the separation of dwarf-b from wild-type sibs became easier as the cultures became older. In order to find the weight of dwarf-b flies as compared with not-dwarf-b sibs, and to determine how these weights changed with the age of the culture, Dichæte Hairless × dwarf-b male-back-crosses were carried out; and the weights of the dwarf-b and of the not-dwarf-b flies were determined for the flies that hatched in successive two-day intervals (table 185). Of the 5 cultures, the first 2 were sisters, the third was of the next generation, and the last 2 were of a third generation. This procedure gave a greater variety of cultural conditions, and made the results more general

than would be the case with 5 sister-cultures. The three sets of data were combined, and were expressed in terms of milligrams per fly in the curves of figure 36. In the first lot of flies to hatch the dwarf-b flies were almost indistinguishable in size from their sibs. Both kinds decreased in size, the dwarf-b more rapidly, to a minimum at the end of 7 days. There was an unexpected secondary rise in both curves to a maximum at about 10 days, and a second

TABLE 185.—*Weights in milligrams of dwarf-b's (above) and Dichæte Hairless sibs (below); days are class centers.*

	dw-b	mg.	dw-b	mg.	dw-b	mg.	dw-b	mg.	dw-b	mg.	dw-b	mg.	dw-b	mg.
Days	1		3		5		7.5		10		12			
12,040.....	29	37.5	22	19.7	21	15.0	17	11.5	11	7.8	4	2.4	...	...
12,041.....	21	25.0	20	19.6	30	25.3	26	22.5	17	15.0	13	10.0	...	...
Days	.5		2		4.5		7		9		11.5		14.5	
12,130.....	5	6.4	27	27.3	20	15.7	13	7.5	6	3.2	8	4.7	11	5.8
Days	1		3		5		7		9.5				13.5	
12,239.....	21	25.0	23	20.8	19	19.7	7	6.2	20	17.3	...	...	10	8.3
12,240.....	13	12.6	27	25.2	27	25.0	19	14.6	7	6.0	...	...	14	8.2
Totals....	89	106.5	119	112.6	117	100.7	82	62.3	61	49.3	25	17.1	35	22.3
Av. days..	.9		2.8		4.9		7.2		9.3		11.8		13.8	
Av. wt....	1.20		.95		.86		.76		.81		.68		.64	
	D H	mg.	D H	mg.	D H	mg.	D H	mg.	D H	mg.	D H	mg.	D H	mg.
12,040.....	30	38.0	29	34.4	32	30.7	14	15.5	17	19.4	9	9.0	...	...
12,041.....	31	38.6	24	30.3	23	23.0	20	23.8	23	28.4	12	13.8	...	...
12,130.....	9	11.5	40	51.0	13	15.6	16	13.7	5	4.5	8	9.5	15	16.4
12,239.....	11	14.2	26	25.1	24	27.0	13	14.7	10	12.6	...	...	18	19.1
12,240.....	9	10.0	30	27.4	25	27.5	23	22.7	17	20.7	...	...	26	25.0
Totals....	90	112.3	149	168.2	117	123.8	86	90.4	72	85.6	29	32.3	59	60.5
Av. wt....	1.25		1.13		1.06		1.05		1.19		1.11		1.03	

falling off to the lowest point at 14 days. The two curves were of similar type, but diverged by the more continuous and more rapid fall of the dwarf-b weights. The secondary rise was present in all the 5 cultures, which cultures belonged to three generations. The cultures were all kept in the incubator at 25° until the offspring began to hatch. The cultures were then removed and kept on a table at room temperature (20° to 25°) during the remainder of the hatching period. It is possible that the secondary rise is connected with this treatment. However, the cultures for the weight curves of dwarf versus not-dwarf (see p. 103, and fig. 36) also treated in this way, and at the same time as the last

two sets above, showed no such rise. The average weight of the non-dwarf-b Dichæte Hairless flies was 1.12 mg., or about 400,000 to the pound. The average weight of the dwarf-b flies in the later counts was about 0.7 mg., or about 650,000 to the pound.

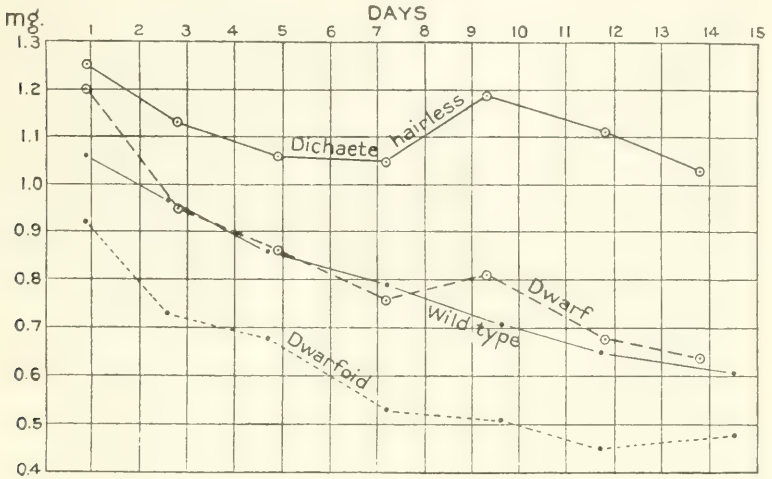


FIG. 36.—Curves of change in weight with age of culture of dwarf and of dwarf-b flies, with the wild-type and Dichæte Hairless controls. (Through errors the curve for the dwarfs is labelled dwarfoid, and the curve for dwarf-b is labelled dwarf.)

EVALUATION OF DWARF-B.

The separations of dwarf-b from not-dwarf-b proved too unreliable, and accordingly the stock of dwarf-b was discarded.

MINUTE-DIII (Mdiii).

ORIGIN AND DESCRIPTION OF MINUTE-D.

Two mutants had been found that were apparently identical somatically. One, forked^2, was known to be an allelomorph of forked, but a less extreme departure from the wild-type than forked is. The other arose later than forked^2 in a culture not related to those in which forked^2 had occurred. It was temporarily called forked-x, the forked denoting its similarity to forked in general characters, and the x denoting unknown genetical relation to forked. The two mutants were crossed together, and it was found that the F_1 females were wild-type in appearance. The two mutants were thus known to be not allelomorphic. Later tests showed that forked-x is an allelomorph of singed, and it was accordingly renamed singed^2. In F_1 of the above cross between forked^2 and singed^2, Bridges found that a very few of the flies had markedly small bristles throughout the body. These bristles were of normal proportions (ratio of thickness to length) and were normal in position and in the directions in which they pointed. The flies were slightly paler in color and slightly

smaller in size, but these characters were not dependable in classification. This character was called a "Minute," from its resemblance somatically to the many "Minutes" previously found. This minute was the ninth found, but was called Minute-5, since it was the fifth found after the Minutes began to be named in series. The name was changed to Minute-d when letters were adopted for series of mimics.

MINUTE × STAR HAIRLESS, MALE BACK-CROSS.

A Minute-d male was out-crossed to a Star Hairless female, preparatory to finding to which chromosome the gene for Minute-d belongs. In F_1 the Minute character was present (culture 11456; +353, M 38), which fact showed that it is a dominant, as were the other mutants that had been called Minutes. Two of the F_1 Star Hairless Minute-d males were out-crossed to wild females, as a male back-cross test (table 186). In the progeny none of the Star flies

TABLE 186.—*P_1, Minute-d ♂ × Star Hairless ♀; B. C., F_1 S H M ♂ × wild ♀.*

March 22, 1920.	S H	S	H	M
11,583.....	64	74	69	63
11,597.....	79	74	78	75
Total.....	143	148	147	138

were Minute, and none of the Hairless flies were Minute, while practically all of the not-Star not-Hairless class were Minutes. The Minutes thus constituted a quarter of the population, and therefore required for their appearance the simultaneous presence of a third- and of a second-chromosome gene, each in heterozygous form.

MINUTE-D × STAR HAIRLESS, FEMALE BACK-CROSS.

Two cultures of the female back-cross (Minute-d × Star Hairless) were carried out (table 187A), for a preliminary location of the two genes with respect to Star and to Hairless. There was apparently 39.7 per cent of recombination for Hairless and Minute-dIII, and 37.7 of Star and Minute-dII (58/146=39.7; 55/146=37.7).

STAR HAIRLESS MINUTE-D × WILD, BACK-CROSS.

Two cross-over Star Minute-dII Hairless Minute-dIII females from 11737 were out-crossed to wild males (table 187B). There was apparently 23 per cent of recombination for Hairless and Minute-dIII, and 38 per cent of recombination for Star and Minute-dII. The Hairless Minute-dIII value is not in agreement with that of the previous experiment.

Three similar cross-over Star Hairless Minute males were out-crossed to wild females (table 187C). In these cultures all of the Star Hairless flies

were expected to be Minute; and they were, with the exception of 8 doubtful specimens. None of the not-Star Hairless flies were expected to be Minute, yet in two cultures nearly half of the Hairless flies were Minute, though of a

TABLE 187A.—P_1, *Minute-d* × *Star Hairless*; *B. C.*, F_1 *S H M* ♀ × + ♂.

May 17, 1920.	+	S	H	S H	M	S M	H M	S H M
11,737	50	49	47	48	37	21	22	11
11,742	36	32	34	45	19	11	13	12
Total	86	81	81	93	56	32	35	23

TABLE 187B.—*Crossover S H M* ♀ × + ♂ (*May 28, 1921*).

11,751	20	26	22	19	9	7	16	22
11,774	50	42	50	32	3	4	10	29
Total	70	68	72	51	12	11	26	51

TABLE 187C.—*Crossover S H M* ♂ × + ♀.

11,752	59	57	35	2	..	..	27	77
11,801	41	42	22	4	..	..	16	32
11,807	49	63	66	2	..	..	..	61
Total	149	162	123	8	..	..	43	170

weaker average grade than the Star Hairless Minutes. It seems probable that in these two cultures some other (sex-linked?) gene was present that acted as an intensifier of Minute-dIII. There had been no previous indication of such action, and it did not appear in the sister cultures.

STAR MINUTE-DII × BLACK PURPLE CURVED, BACK-CROSS.

The next points to be determined were the locations within the second and third chromosomes of the two Minute genes. To locate Minute–dII, Star Hairless Minute-d males were out-crossed to black purple curved females

TABLE 188.—P_1, *Star Hairless Minute-d* ♂ × *black purple curved* ♀.

May 20, 1920.	+	H	S b!	S b! H M
11,757	28	32	37	26
11,803	61	55	53	41
Total	89	87	90	67

(table 188). Roughly, a quarter of the offspring were Minutes, namely, all those that were at the same time Star and Hairless. But, unexpectedly, all the

flies that were Star were at the same time black. No black was known in the ancestry of Minute. One of the F_1 Star black Hairless Minute males was back-crossed to black purple curved females, with the result that there were 109 b pr c : 62 S b ! : 60 S b ! H M, or a 2 : 1 : 1 ratio, as expected. The female back-cross (table 189) showed that the locus of Minute-dii is between

TABLE 189.—*B. C., F_1 Star-black ! Hairless Minute-d* ♀ (*11,803*) *× b pr c* ♂.

Aug. 14, 1920.	b pr c	b pr	b c	b	b pr c M	b pr M	b c M	b M
11,896.....	106	11	14	69	1	8	3	26
11,897.....	89	4	27	38	1	15	2	34
Total..	195	38	46	167	2	23	5	60

those of purple and curved, and about 4.5 units to the left of curved, *i. e.*, at about 69. This location is not very accurate. The character is not useful enough to warrant more work.

HAIRLESS MINUTE-DIII × SEPIA, BACK-CROSS.

Similarly, to locate Minute-diii within the third-chromosome, a Star Hairless Minute male was out-crossed to a sepia female, and F_1, Star Hairless Minute males were back-crossed to sepia males (table 190). There was

TABLE 190.—*P_1, Hairless Minute-d* ♂ (*11,807*) *× sepia* ♀; *B. C., F_1 H M* ♀ *× se* ♂.

Aug. 3, 1920.	se	+	se H	H	se M	M	se H M	H M
11,907.....	17	14	8	10	7	5	8	12
11,909.....	78	28	15	36	20	8	36	45
11,926.....	8	1	2	4	2	..	4	9
11,931.....	69	49	19	36	12	10	42	43
Total..	172	92	44	86	41	23	90	109

24.3 per cent of recombination for Hairless and Minute, and the locus of Minute-diii was shown to be to the right of those for sepia and Hairless. The above Hairless Minute-diii data were combined with those previously obtained and the gross recombination per cent (24.0) was compared with those for Hairless and various other mutants such as rough, Pointed-wing, claret, and Minute. The locus of Minute-diii was inferred to be slightly to the right of rough, or at about 95. This location is uncertain, though, as in the case of Minute-dii, the region in which the gene is supposed to lie contains many good loci by means of which more accurate determinations may be carried out if it should be thought advisable.

ROUGH^2.

Muller reports that a more extreme allelomorph of rough (called rough^2) arose in some linkage experiments that he was carrying out (March 1920). This mutant offered no advantages over the previously known mutant, and it was not retained.

DELTA-B.

ORIGIN AND DESCRIPTION OF DELTA-B.

Among the F_1 flies from the cross of Star Hairless Minute-d to sepia white-ocelli (see p. 234), appeared one Star male that had characteristics resembling those of the third-chromosome dominant Delta (culture 11874, August 13, 1920). The wings were small and were held out at a wide angle from the body. The veins were slightly branched and knotted, and were confluent at the margins. The dorsal hairs were more numerous than normal and the rows were irregular. The bristles were strong, and there were supernumerary anterior postalars. The body-color was dark as in Delta, and the eyes were extreme Star (like intensified Star of p. 175). The third legs were shortened as in bent. The characters of "Delta-b" were more extreme than those of Delta, and there were other definite differences, the most striking of which was that the venation was of the confluent type rather than of the simple Delta type.

DELTA-B A DOMINANT IN CHROMOSOME-III.

From the fact that the character Delta-b had occurred as a single fly in the offspring of a pair culture, it seemed probable that the character was a dominant. In that case its location in the second or the third group could be determined directly by out-crossing the Star Delta-b male to a sepia white-ocelli female. The mother of this male had been sepia white-ocelli, so this mating constituted a male back-cross for the second-chromosome (Star) and for the third (se wo). In the offspring of the above mating (culture 11929) there were 4 flies that were Delta-b, and these were at the same time sepia and white-ocelli. Several other dead Delta-b's were found in the bottle, and also several dead pupæ that were probably Deltas. Besides these Deltas, there were no flies that were sepia white-ocelli; that is, the gene is a dominant in the same chromosome with sepia and white-ocelli. Nearly all the flies possessing the character die. There were 73 not-Deltas that were Stars and not-Stars.

None of the four Delta-b flies were fertile, hence the mutant could not be maintained.

DARK (dk).

In order to determine whether mutations similar to those found in the laboratory cultures were occurring among the wild-flies of *Drosophila melanogaster,* Dr. R. E. Clausen set traps at various places about Berkeley, and then raised offspring from the wild flies, to see whether new forms would be present in the first or second generations. In one case three new forms appeared, viz. a second-chromosome recessive "cinnabar," located about 3 units to the right of purple, a sex-linked sex-limited recessive similar to or identical with the mutant "bobbed," and a "dark" eye-color, similar in appearance to saffranin or to (not-eosin) cream–III (September, 1920). This dark eye-color proved

to be a recessive, and gave in male back-crosses with Star Dichæte the 1 dk: 1 S dk: 1 D: 1 S D ratio typical of a third-chromosome mutant. The locus within the third-chromosome has not yet been determined. Clausen reports that the character can not always be distinguished with certainty from the wild-type. Perhaps the use of eosin or of vermilion as a "sensitizer" (see cream–III, p. 112) might make the mutant workable. In that case, the usefulness of the mutant would largely depend on whether its locus were in a region not already well represented by other satisfactory mutants.

MINUTE–F (Mf).

In working with the eleventh member of the series of allelomorphs and remutations of cut, Bridges found that about a third of the flies of one culture had small bristles and other characteristics belonging to the type of mutant called "Minute" (Sept. 9, 1920, 11974; M 16, +25). This particular Minute was No. 18 in the numbered series (about the twenty-fifth found) and hence was called Minute-18. Later the name was changed to Minute-f, which is used in the following account. In an out-cross to sepia white-ocelli, Minute-f appeared in F_1 as a dominant. It was delayed in emergence and poorly viable, as is the case with other Minutes (11993, M 49, +94). Several female back-crosses with sepia white-ocelli failed to produce more than a few flies, indicating, however, that the locus of Minute is in the third-chromosome to the right of that of white-ocelli. A sepia white-ocelli Minute fly from the back-cross was out-crossed to wild, and a single back-cross culture raised (table 191).

TABLE 191.—*P_1, sepia white-ocelli Minute-f ♂ × wild ♀; B. C., F_1 Mf ♀ × se wo ♂.*

Dec. 27, 1920.	se wo Mf	+	se	wo Mf	se wo	Mf	se Mf	wo
12,178.....	44	37	39	37	19	25	17	11

There was 31.5 per cent of recombination for white-ocelli and Minute-f. The locus of Minute-f is probably not far from the right end of the map of the chromosome, and has been rather arbitrarily placed at 105.0.

Some further work on Minute-f was done; but so much sterility was encountered that the stock was soon thrown away.

MINUTE-G (Mg).

In working with Minute–dIII, Bridges found that one strain differed from the others in that the Minute character was easily separable from the not-Minutes, and was present in out-crosses in 1:1 ratios rather than in only a quarter of the offspring. It was supposed that a new Minute (called Minute-23, here called Minute-g) had arisen in a strain of the old Minute. In out-crosses the effect seen would be due to the new Minute, for Minute–dIII was known to require the simultaneous presence of a second-chromosome dominant, and the out-crosses should have eliminated this dominant, at least from many of

the cultures. The strain was maintained by continually back-crossing Hairless Minute-g males by claret females. Such male back-crosses gave an approximate equality of Hairless Minute and claret offspring (*e. g.*, culture 12013, H M 54, ca 57).

HAIRLESS MINUTE-G × CLARET, FEMALE BACK-CROSS.

Female back-crosses were made parallel to the stock cultures to find the position of the Minute gene. Counts of four generations of such cultures are given in table 192. The locus of Minute-g is definitely to the right of claret,

TABLE 192.—*P_1 Hairless Minute-g × claret; B. C., F_1 H Mg ♀ × ca ♂.*

Sept. 27, 1920.	H Mg	ca	H ca	Mg	H	ca Mg	H ca Mg	+
11,990	4	3	1	2	1	2	..	1
11,986	6	8	2	7	..	..	..	..
12,082	104	74	53	41	7	5	2	1
12,118	71	69	37	22	4	7	..	2
12,119	90	66	27	33	8	..	..	6
12,120	27	29	21	10	5	3	2	..
12,121	71	59	33	33	6	3	1	1
12,122	53	57	27	23	1	3	..	..
12,123	67	53	18	18	4	5	..	..
12,148	38	37	17	21	7	1	..	2
12,149	75	79	32	43	6	2	1	3
12,150	24	34	10	16	2	..	..	..
12,151	63	61	30	30	5	2	..	1
Total	693	629	308	299	56	33	6	17

and is the locus farthest to the right of those thus far discovered. There was 5.5 per cent of recombination for claret and Minute.

A remarkable feature of the data is the high frequency of the double-recombination classes. The coincidence is 67, as compared with a coincidence of about 15 for the same section between Hairless and claret, judged from other data.

Minute-g has not been worked with except in the above experiment, hence its relation to Minute–dIII has not been investigated, nor was its position well determined by this single experiment. Classification seemed sharp; but there was seen to be an inequality in the parallel classes H and ca M and also H ca M and + that may be explained as overlap of Minute into wild-type. This is not certainly the case, and the results are not to be relied upon completely. The locus is at 106.2, taking the data at their face value.

VARNISHED (vr).

Through the courtesy of Dr. O. L. Mohr we include the following summary of his discovery and breeding-work with the third-chromosome recessive "varnished." Two mutant males were found, October 22, 1920, in a stock of the second-chromosome dominant Gull. The eyes are smaller than normal, with irregular surface of glassy "varnished" texture. The wings are often

slightly curved, and may have a short fifth vein. The females were found to be sterile, but the males were of normal fertility. A back-cross with Delta gave 40 vr, 53 Δ, 11 vr Δ, 17 +, corresponding to 23.1 per cent of recombination. Four cultures of the back-cross of varnished to Dichæte Hairless gave the order of loci, Dichæte varnished Hairless, with 4.2 per cent of recombination for Dichæte and varnished and 28.9 for varnished and Hairless (table 193).

TABLE 193.—*P_1, varnished × Dichæte Hairless; B. C., F_1 D H ♀ × vr ♂ (O. L. Mohr).*

Dec. 13, 1920.	D H	vr	D vr	H	D	vr H	D vr H	+
2,363 ff	230	192	10	7	105	65	3	6

The Dichæte Hairless per cent was 30.3, which is higher than the standard D H per cent of 25. If the increase above normal is uniform throughout the intermediate region, then the 4.2 may be corrected to 3.6. The locus of varnished may be placed provisionally at 3.6 units to the right of Dichæte, or at 44±. The relative order of varnished with respect to the numerous other mutants in that region must be determined by special experiments.

POINTED-WING (Pw).

(Figure 37.)

ORIGIN AND DESCRIPTION OF POINTED-WING.

In carrying on a stock that was producing intersexes because of the previous occurrence of triploidy, Bridges found a few flies with pointed wing-tips (12625, March 29, 1921). The third and fourth longitudinal veins tend to approach each other, and have a cross-vein, or plexus of small branch-veins, near the tip of the wing. There were a few other slight changes, such as a tendency for the posterior scutellar bristles to stick up. In classification the vein abnormality was found the most convenient characteristic.

THE ANTENNA-WING CHARACTER OF POINTED.

In some cultures a rather large proportion of the Pointed-wing flies were seen to have antennæ that were much modified, apparently in the direction of wings (see fig. 37b). The antenna had grown out into a structure much like the extra-wings of bithorax, *i. e.*, there was present a distinct marginal vein with a break in it, as at the base of a normal wing, and with rows of heavy hairs or bristles along it. The rest of the surface had very tiny hairs as do the surfaces of wings. The modification of the antenna in the direction of a wing is of interest, because previously a mutant had been found by Bridges in which the antennæ were distinctly leg-like in character with a series of fine distinct cylindrical joints armed with bristles and with two strong curved bristles as in a normal foot.

POINTED × POINTED; DOMINANT, LETHAL WHEN HOMOZYGOUS.

Three pairs of Pointed-wing flies were mated, and in F_1 there was a total of 108 Pointed-wings to 60 that were wild-type (12685; Pw 18, +13; 12686, Pw 59, +33; 12687, Pw 31, +14). The wild-type flies were not genetically the same as the Pointed, as was shown by the distinctness and by out-crosses of the classes. The Pointed-wing flies were then heterozygous dominants; and that the homozygous Pointed-wing is lethal was indicated by the close approach

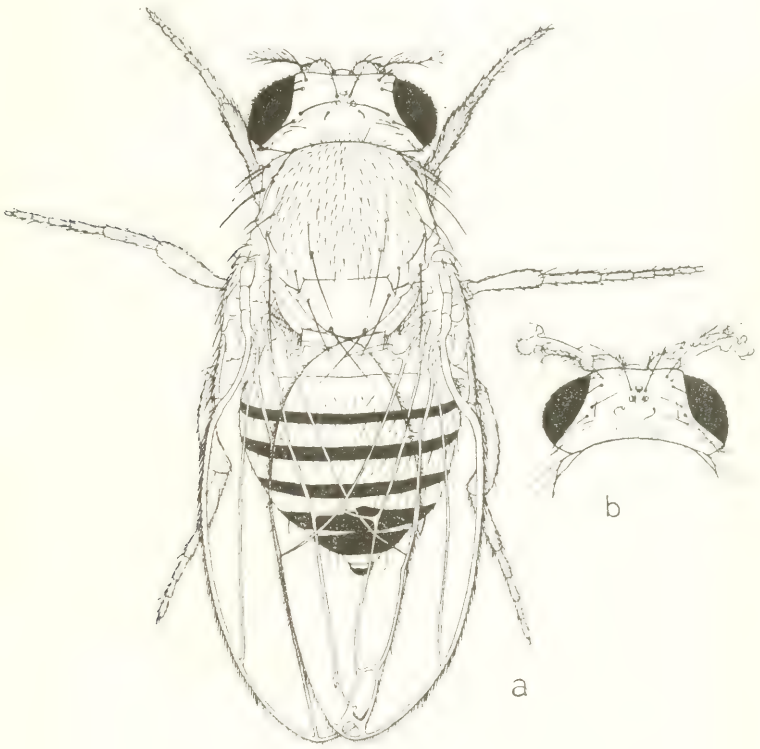


FIG. 37.—(*a*) Pointed-wing; (*b*) antenna-wing of Pointed.

to a 2:1 ratio rather than a 3:1 ratio. Continued inbreeding of Pointed failed to produce a pure-type stock. The lethal nature of the homozygous Pointed was ultimately proved by the fact that a balanced pure-type stock was secured by mating Pointed to CIII IIIa and inbreeding the double heterozygote.

POINTED-WING × DICHÆTE.

Pointed-wing was observed to be distributed at random with respect to sex, and with respect to the second-chromosome recessives plexus and speck. Pointed-wing was therefore assumed to be in the third-chromosome, which assumption was proved correct by the results of Dichæte × Pointed male back-crosses (12870, D 68, Pw 72; 12872, D 52, Pw 51). A single D × Pw female

back-cross gave free recombination of Dichæte and Pointed (12892, June 9, 1921, D 11, Pw 8, D Pw 3, +12; the per cent of recombination was 44.1).

POINTED-WING × SOOTY CLARET.

To see whether or not the locus of Pointed is far to the right of Dichæte, a Pointed by sooty claret back-cross was carried out (table 194). The locus

TABLE 194.—P_1, *Pointed-wing × sooty claret*; *B. C.*, F_1 *Pw* ♀ × e^s *ca* ♂.

June 30, 1921.	e^s ca	Pw	e^s Pw	ca	e^s	Pw ca
12,950.....	33	42	13	13	3	4
12,951.....	27	13	8	3	3	3
12,952.....	11	15	1	4	..	3
Total.....	71	70	22	20	6	10

of Pointed proved to be between those of sooty and claret. There was 8.0 per cent of recombination for Pointed-wing and claret.

POINTED-WING × EBONY^4 ROUGH.

The locus of Pointed-wing would seem to be near that of rough, which gives about 10 per cent of recombination with claret. Since Pointed is a dominant, this relationship is easy to test by direct back-cross. Accordingly, a Pointed-wing claret female was out-crossed to an ebony^4 white-ocelli rough male, and F_1 Pointed-wing females were again crossed to e^4 wo ro males. It was found that all the F_1 Pointed-wing flies were also white-ocelli, which indicates more exactly the origination of the Pointed-wing mutation, for the intersex stock was known to be heterozygous for white-ocelli. The character white-ocelli had never been seen in the Pointed-wing flies because of its recessiveness, the lethal nature of homozygous Pointed, and the fact that the two loci are so close together that crossing-over is not frequent. In the back-cross of table 195 the character white-ocelli was present throughout.

TABLE 195.—P_1, *Pointed wing* [*claret*] ♀ × *ebony*^{y} [*white-ocelli*] *rough* ♂; *B. C.*, F_1 *Pw* ♀ × e^y *wo ro* ♂.

July 22, 1921.	e ro	Pw	e Pw	ro	e ro Pw	+	e	ro Pw
12,970.....	104	121	33	34	8	3	..	..
12,971.....	100	113	26	36	7	2	..	..
12,972.....	115	118	34	34	2	9	..	2
12,973.....	91	109	28	31	4	3	..	..
Total.....	410	461	121	135	21	17	..	2

The locus of Pointed proved to be to the right of that of rough: and rough and Pointed gave 3.4 per cent of recombination.

EBONY[4] ROUGH × POINTED-WING CLARET.

A stock of ebony[4] (we) rough claret was obtained, and a four-point back-cross from the mating of ebony[4] rough by Pointed-wing claret was carried out (table 196). The rough Pointed-wing recombination per cent was 3.2.

TABLE 196.—P_1, *Pointed-wing claret × ebony[4] rough; B. C., F_1 Pw ♀ × e[4] ro ca ♂.*

Aug. 14, 1921.	e[4] ro	Pw ca	e[4] Pw ca	ro	e[4] ro Pw ca	+	e[4] ro ca	Pw	e[4]	ro Pw ca
12,998.....	82	99	29	29	2	5	11	10	1	..
12,999.....	111	117	19	31	5	2	10	11	..	..
13,000.....	85	92	23	30	7	1	11	9	..	1
13,001.....	92	87	18	29	6	5	14	6	..	..
Total....	370	395	89	119	20	13	46	36	1	1

which agrees with the 3.4 of the previous experiment. The Pointed-wing claret per cent was 7.5, which agrees with the 8.0 of the Pointed × sooty claret experiment.

The primary base of reference of Pointed is rough; and Pointed lies about 3 units to the right of rough, according to the weighted mean of the different sets of determinations, or at 94.1.

EVALUATION OF POINTED-WING.

The separability of Pointed-wing is fairly good but not excellent. Its viability and other characteristics of breeding behavior are excellent, and its dominance makes its usefulness rise nearly to a par with that of the mutant rough, for which it may be substituted in a fair proportion of experiments.

SHRUNKEN (WZ).

In studying the ratios in which the fourth-chromosome recessive eyeless appeared in various matings in which an extra fourth-chromosome was involved, Bridges observed that many of the flies were small and shrunken in appearance (June 1921). The thorax was depressed, and the body and abdomen were not filled out. The chitinization was weak. The body-color was dark and had a dull translucent tone. The bristles were small. The flies were much delayed in hatching.

Most of the "shrunken" flies had at the same time pink eyes, which character had been present in the eyeless stock. Because of the close association of shrunken with pink, the locus of shrunken was known to be in the third-chromosome.

SHRUNKEN PINK × DICHÆTE.

A shrunken pink male was crossed to a Dichæte female; and three pairs of F_1 Dichæte flies were mated (table 197A). Also, two back-cross cultures

were raised (table 197B). There were 2 flies in which the characters pink an shrunken had undergone recombination, and these showed that the locus shrunken is between those of Dichæte and pink. The recombination per cen

TABLE 197A.—P_1, *shrunken pink* ♂ × *Dichæte* ♀; F_2, F_1 *D* ♀ × F_1 *D* ♂.

July 23, 1921.	D	wz p	D wz	+	D p	wz
12,974.....	221	112	7	8	..	..
12,975.....	209	109	7	3	..	..
12,976..	244	102	6	5	1	..
Total ...	674	323	20	16	1	..

TABLE 197B.—*B. C.*, F_1 *D* ♀ × *wz p* ♂.

12,995 . . .	85	58	5	4	..	..
12,997.....	64	52	3	3	..	1
Total .	149	110	8	7	..	1

for shrunken and pink was 0.2; that is, the locus of shrunken is two-tenths of a unit to the left of that of pink or at 47.8.

The stock of shrunken was discarded, owing to the difficulty encountered in handling the mutant.

LETHAL-IIIH.

In changing the method of keeping cut[18], Miss Phoebe Reed mated a cut[18] male to Star Dichæte female, and bred together the S D offspring. The progeny (disregarding cut[18]) were 144 S D, 10 S, 31 D, 7 +; that is, there were far too few not-S and not-D flies (November 28, 1921). To account for these ratios it was assumed that both a second-chromosome lethal and a third-chromosome lethal were present, having been introduced from the cut[18] grandparents. The Star lethal–H recombination per cent was 37.5. The second-chromosome lethal was not tested further. The total of 17 not-D flies (recombinations) and 17.5 D flies (2 *oc* + 1 *rc*) correspond to a recombination per cent of 17.7.

LETHAL-IIIH × HAIRLESS.

To discover whether the locus of the lethal were to the left or to the right of Dichæte, a Dichæte male from the above culture was out-crossed to a Hairless female. Three pairs of F_1 not-D H flies were inbred (table 198). All gave ratios in which there were markedly more Hairless than the 67 per cent expected on a non-lethal basis. In total the 126 wild-type flies corresponded to 48.9 per cent of recombination. That is, the locus of the lethal was so far from that of Hairless as to be unquestionably to the left rather than to the right of Dichæte.

TEST FOR SERIAL ORDER OF LIIIH AND HAIRY; LETHAL-III I.

The liiih–D recombination per cent of 17.7 suggested that the locus of liiih is to the left of that of hairy, for the standard h-D recombination is

per cent. An experiment was planned to test this serial order as follows: From table 198, several Hairless males, about two-thirds of which should

TABLE 198.—P_1 (*D over lethal-*lllh) ♂ × *Hairless* ♀: F_2. F_1 (*H over* lllh) ♂ and ♀.

March 10, 1922.	H	+
457	83	23
457r	157	55
460	149	48
Total	389	126

have lllh in the not-H chromosome, were out-crossed to se h e^s females, and several F_1 ♀ daughters were then crossed singly to se h e^s males. It was intended to take the he^s flies that were the result of crossing over between se and e^s and test them for the presence of lllh. Unfortunately, only two of the many cultures produces offspring, and in these only one se e^s recombination appeared (table 199). This female was crossed to a sepia white ocelli

TABLE 199.—P_2. *Hairless lethal-*lllh ♂ × *sepia hairy sooty* ♀: F_1 ♀ × *se h e^s* ♂.

April 24, 1922.	se h	—	se	h
13,127	51	67		
13,134	129	104	1	
Total	180	171	1	

and four pairs of the not-se flies were inbred (table 200). In none of 10 cultures was a lethal present with a locus to the left of hairy, from which we may conclude either that the original cross-over fly was one of the

TABLE 200.—F_2 *from* lllh ? *h e^s* × *se wo*.

July 5, 1922.	h e^s	h	e^s	se wo	se	wo	+
13,177	2	21	5	56	24	34	110
13,185	20	11	21	23	29	18	54
13,186	44	27	28	41	39	26	83
13,187	35	28	29	54	31	22	90

minority expected to be without the lethal, or that the locus of lllh is not to the left of hairy. The stock has now been lost, so that this must remain unsettled.

But one of the cultures (13177) showed the presence of a new lethal betwe hairy and sooty and very near sooty. This lethal is certainly one introduc through the se h e[3] stock used originally. An account of this lethal (lII appears in a later section.

MINUTE-H (Mh).

In late November 1921, Miss Phoebe Reed found a fly that was an interse It had ovaries and female genitalia, but its coloration and the shape of abdomen were male-type, and it showed a rudimentary sex-comb on one tarsu This intersex bred as a female, giving only normal offspring in F_1 and in I But in one of the F_2 cultures a male appeared (December 20, 1921), one whose wings was smaller than normal, and whose bristles were "Minute" o the half of the thorax corresponding to the small wing.

This mosaic male was out-crossed to a yellow female whose two X–chrom somes were joined to one another (resulting in 100 per cent non-disjunction and approximately half of the offspring were found to show the Minute-brist character (y ♀ 59, y M ♀ 57, + ♂ 49, M ♂ 46). The Minute appeared in males as well as in F_1 females, and was thus known to be an autosom dominant.

The Minute-bristle character had not been present in either parent, and w only present in part of the first representative of the race. The cells of tl testes were heterozygous for the mutant. The mutation occurred before tl germ-cells arose. There has been a score or more of such mosaics by mutatio in an early cleavage stage, but few of them have been bred from.

MINUTE-H × STAR DICHÆTE; MINUTE-H × CURLY HAIRLESS.

To discover to which linkage group the gene for Minute-h belongs, Minu was crossed to Star Dichæte; and an F_1 S D M male was out-crossed to a wil female. None of the Minute offspring were Dichæte, while half were St (D 61, S D 38, M 5, S M 7). Minute is thus shown to be a member of th third-chromosome group. A similar back-cross of Minute × Curly Hairle gave the same type of result (H 38, Cy H 40, M 17, Cy M 8).

STRIPE (sr).

While selecting some flies from the Oregon stock of wild, Bridges foun that there were present in the stock several individuals, both females an males, with a dark stripe down the thorax (Feb. 6, 1922). The character w very similar in appearance to the second-chromosome dominant Streak. Th pigment of the stripe seemed to lie deeper in the thorax than does that o the trident, which is epidermal. There was the same flattened appearance t the thorax as in Streak, and the thorax frequently contained bubbles. Th character was, on the average, considerably more pronounced than is Streal Some of the stripe flies were mated together, and all the offspring possesse stripes.

STRIPE × STAR DICHÆTE, BACK-CROSS.

A stripe male was out-crossed to Star Dichæte female. None of the offspring were stripe. F_1 S D females were back-crossed to stripe males (table 201). The characters Star and stripe were assorted at random. There was

TABLE 201.—*Stripe ♂ × Star Dichæte ♀; B. C., F_1 S D ♀ × sr ♂.*

Mar. 3, 1922.	D	S D	sr	S sr	D sr	S D sr	+	S
13,103.....	83	71	83	80	15	22	31	23
13,104.....	49	41	41	28	9	10	9	6
13,105.....	73	86	69	67	20	11	11	7
Total..	205	198	193	175	44	43	51	36

linkage of Dichæte and stripe, with 174 recombinations in the total of 945, corresponding to 18.4 per cent of recombination.

STRIPE × DICHÆTE HAIRLESS.

The next experiment with stripe was the three-point back-cross of stripe × Dichæte Hairless, to determine the relative order of the loci stripe and Dichæte (table 202). In working with the mutant Streak, it had been observed that

TABLE 202.—*P_1, Dichæte Hairless × stripe; B. C., F_1 D H ♀ × sr ♂.*

April 22, 1922.	D H	sr	D sr	H	D	sr H	D sr H	+
13,123.....	87	101	23	29	13	17	1	2
13,124.....	113	117	28	41	12	14	6	2
13,128.....	41	21	6	9	7	3	..	2
13,129.....	158	158	56	44	18	27	3	2
Total....	399	397	113	123	50	61	10	8

the presence of the dark body-color black, instead of interfering with the classification of Streak, make it considerably easier. Accordingly, all the flies of the stripe × Dichæte Hairless back-cross were made homozygous for black. No difficulty had been met with in making complete separations for stripe, so that the above modification was primarily for a comparison of Streak and stripe. The locus of stripe was found to be to the right of Dichæte. The percentage of Dichæte stripe recombination was 21.9, somewhat higher than the 18.4 of the previous table.

SPINELESS STRIPE, BACK-CROSS.

The stripe Hairless recombination per cent in the DH × sr back-cross of table 202 was 11.1, which is the same as the standard spineless Hairless per cent of 11.0. The locus of stripe is accordingly very close to that of

spineless. But the Dichæte stripe recombination per cent was 21.9, which is higher than the standard Dichæte spineless recombination of 15.7. If the 11.1 is correspondingly above standard, then the locus of stripe is less than 11 units to the left of Hairless, or is probably to the right of spineless by 2 or 3 units.

A test of this hypothesis as to the location of stripe was made by crossing stripe to spineless bithorax, and back-crossing the F_1 females by spineless bithorax (table 203), then crossing these spineless not-bithorax flies to stripe.

TABLE 203.—P_1, *stripe* × *spineless bithorax*; F_1 + ♀ × *ss bx* ♂.

May 23, 1922.	ss bx	+	ss	bx
13,147	111	96	1	..
13,148	120	113	..	..
13,149	95	107	1	..
13,150	117	139	..	..
13,154	143	192	..	..
13,155	142	196	1	..
13,156	123	135	1	..
13,157	157	182	..	..
Total	1,008	1,160	4	0

If the locus of stripe is to the right of that of spineless, it is likely to be also to the right of that of bithorax, which is itself only 0.2 unit to the right of spineless. Accordingly, every spineless not-bithorax back-cross fly should have bithorax replaced by stripe. The tests showed that this was the case.

A stock of spineless stripe was secured by breeding together the stripe offspring (ss sr/sr) that came from the test matings of spineless not-bithorax B. C. flies to stock stripe. A spineless × stripe back-cross was made (table 204) which showed 3.7 per cent of recombinations for spineless and stripe. The

TABLE 204.—P_1, *spineless bithorax* × *stripe*; *B. C.*, F_1 + ♀ × *ss sr* ♂.

July 15, 1922.	ss	sr	ss sr	+
13,188	191	191	9	9
13,189	213	236	5	10
13,190	145	154	7	7
13,191	232	244	8	6
Total	781	825	29	32

locus of stripe is not far from that of glass, for the standard recombination for spineless and glass is 4.6.

SPINELESS STRIPE × DELTA HAIRLESS.

Since the D-H recombination per cent in the sr × D H back-cross had been higher than standard, a ss sr back-cross was made in which Delta and Hair-

less were present as a check upon the kind of crossing-over taking place (table 205). The recombination for ss and H was 11.1, which is the same

TABLE 205.—*P_1, spineless stripe × Delta Hairless; B. C., F_1 Δ H ♀ × ss sr ♂.*

Aug. 12, 1922.	ss sr	ΔH	ss Δ H	sr	ss sr ΔH	+	ss sr H	Δ
13,224.....	115	141	2	4	7	8	4	6
13,225.....	168	170	7	6	5	11	9	1
13,226.....	133	133	2	4	11	10	4	1
13,227.....	169	204	11	11	12	12	4	2
Total....	585	648	22	25	35	41	21	10

as the standard 11.0. The ss-sr recombination was 3.4, the sr–Δ 5.5 and the Δ–H 2.2. The Δ–H value is low, and this is at least partly due to the fact that the Delta character is a more extreme departure from wild-type and is less viable than is the Δ H combination.

EVALUATION OF STRIPE.

The locus of stripe is 3.5 units to the right of spineless, at 62.0, which is close to the locus of glass. But the character stripe is one that does not interfere with the classification of any other character, while glass interferes with eye-colors and eye-structure characters. Stripe is particularly easy to use with sooty, for the presence of the sooty character emphasizes the stripe.

CRUMPLED (cm).

In looking over the Dichæte Hairless stock for virgin DH females, Bridges found a few males and females with small crumpled wings (April 2, 1922). The size of the wing and the amount of the crumpling were both quite variable, but it was found that the two bristles at the tip of the scutellum diverge somewhat from one another and are stubbier than normal. The bristle character seems to be a safe criterion of the mutant.

DICHÆTE HAIRLESS × CRUMPLED.

A crumpled male was mated to a DH sister; and an F_1 DH ♀ was mated to this same male (table 206). The locus of crumpled proved to be in the third-

TABLE 206.—*P_1, crumpled ♂ × Dichæte Hairless ♀; B. C., F_1 D H ♀ × cr ♂.*

April 22, 1922.	D H	cm	D cm	H	D H cm	+	D H	cm
13,125.....	104	91	38	35	41	57	9	5

chromosome, to the right of both D and H. The H cm recombination per cent was 29.5 which would place crumpled to the right of rough.

ROUGH CLARET × CRUMPLED

To find whether the locus of crumpled was to the right or to the left of claret, a crumpled male was crossed to a rough claret female, and F_1 wild-type females were crossed to rough claret males (table 207). Cross-over claret flies

TABLE 207.—*P_1, crumpled ♂ × ebony⁴ white-ocelli rough claret ♀; F_1 +♀ × ro ca ♂.*

May 24, 1922.	ro ca	+	ro	ca
13,151	108	136	10	19
13,152	94	107	12	15
13,153	108	101	12	23
Total	310	344	34	57

were then tested by crossing to crumpled, and since several gave crumpled in half the offspring, the locus of crumpled is known to be to the left of that of claret. By inbreeding the crumpled flies from the test-cultures, crumpled claret double recessives were obtained. Similarly cross-over rough flies were tested by crumpled, and since some of these also gave crumpled progeny in the test cultures, the locus of crumpled is known to lie to the right of rough. That is, crumpled lies between rough and claret. By inbreeding the crumpled offspring of this test a rough crumpled stock was obtained.

These two stocks were crossed together, and the F_1 crumpled females were crossed to rough claret males (table 208). The rough claret recombinational

TABLE 208.—*P_1, ebony⁴ white-ocelli rough crumpled ♂ × crumpled claret ♀; F_1 +♀ × e⁴ wo ro ca ♂.*

July 7, 1922.	ro	ca	ro ca	+	Continued.	ro	ca	ro ca	+
13,178	106	104	11	10	13,181	68	59	13	7
13,179	122	143	11	15	13,182	126	115	18	22
13,180	100	72	12	10					
					Total	522	493	65	64

flies were bred together, and approximately a quarter of their progeny were triple recessives, ro cm ca.

TABLE 209.—*P_1, rough claret × crumpled; B. C., F_1 +♀ × ro cm ca ♂.*

Aug. 17, 1922.	ro ca	cm	ro cm	ca	ro	cm ca
13,230	124	109	6	2	6	12
13,231	176	140	..	4	11	11
13,232	25	27	..	1	2	2
Total	325	276	6	7	19	25

A three-point backcross $\left(\frac{aa\ b\ cc}{+\ +\ +}\right)$ was then made (table 104) to obtain it was found that the recombinations for *a* and *cc* was 14, and for *b* and *cc* was 8.7. The locus of *arsenfed* is thus slightly less than 2 units to the right of *aright*, or at 10.0.

LETHAL-GIII (*lg*)

In looking for the serial order of the last trait and locating *Delight* band and one culture, 31177, July 3, 1930, was carrying a new lethal (*lg*) situated between *hairy* and *curly*, rather near to *curly*. This line was considered to breeding together, in pairs, the wild-type offspring in culture 31177. All four pairs (table 105) showed the presence of this *lethal* factor, the white-eyed

TABLE 105.—*First matings of wild-type flies from 31177, lethal (lg) group*

Mating No.	Sex	♀	♂	Total	♀	♂
31-101	♀	46	0	46		46
	♂	0	—	46	—	0
31-102	♀	52	0	52	52	0
	♂	0	52	52	0	52
Total		98	52	150	98	52

by crossing-over. These data on the position of *lethal* are combined with the similar data of culture 31177 to give a total of $+ = 97.1$, $b = 2.9$, $c = 2.9$, $b\ c = 0$. From these are calculated a *b*-*lethal* recombination per cent of 5.8 and a *lethal*-*c* recombination per cent of 5.8. The locus of *lethal* is thus at about 8.0.

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